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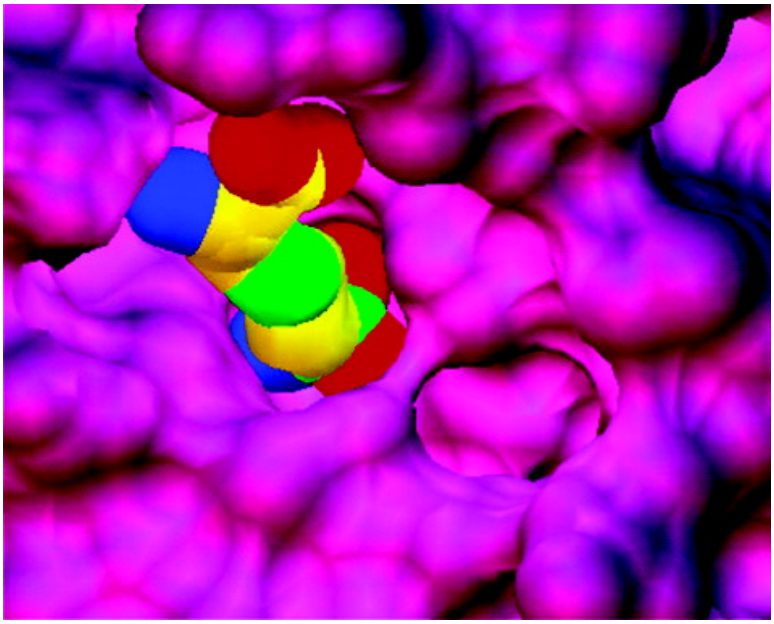

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Design of Amino Acid Sulfonamides as Transition-State Analogue Inhibitors of Arginase

Evis Cama, Hyunshun Shin, and David W. Christianson*

Contribution from the Roy and Diana Vagelos Laboratories, Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323

Received May 27, 2003; E-mail: chris@xtal.chem.upenn.edu

Abstract: Arginase is a binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to form L-ornithine plus urea. Chiral L-amino acids bearing sulfonamide side chains have been synthesized in which the tetrahedral sulfonamide groups are designed to target bridging coordination interactions with the binuclear manganese cluster in the arginase active site. Syntheses of the amino acid sulfonamides have been accomplished by the amination of sulfonyl halide derivatives of (*S*)-(*tert*-butoxy)-[(*tert*-butoxycarbonyl)-amino]oxoalkanoic acids. Amino acid sulfonamides with side chains comparable in length to that of L-arginine exhibit inhibition in the micromolar range, and the X-ray crystal structure of arginase I complexed with one of these inhibitors, *S*-(2-sulfonamidoethyl)-L-cysteine, has been determined at 2.8 Å resolution. In the enzyme−inhibitor complex, the sulfonamide group displaces the metal-bridging hydroxide ion of the native enzyme and bridges the binuclear manganese cluster with an ionized NH^- group. The binding mode of the sulfonamide inhibitor may mimic the binding of the tetrahedral intermediate and its flanking transition states in catalysis. It is notable that the ionized sulfonamide group is an excellent bridging ligand in this enzyme−inhibitor complex; accordingly, the sulfonamide functionality can be considered in the design of inhibitors targeting other binuclear metalloenzymes.

Introduction

Arginase is a binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-arginine through a metal-activated hydroxide mechanism to form L-ornithine plus urea.[1−3] This comprises the final cytosolic step of the urea cycle in liver, where arginase I is predominantly localized.[4] In contrast, arginase II is localized in nonhepatic tissues and plays a role in L-arginine homeostasis, regulating L-arginine or L-ornithine pools for subsequent biosynthetic transformations.[3] Consistent with a role in L-arginine homeostasis for nonhepatic arginase, arginase inhibitors enhance NO-dependent relaxation in gastrointestinal[5] and penile[6,7] smooth muscle tissues, presumably by increasing the bioavailability of substrate L-arginine for NO biosynthesis. To date, arginase inhibition is the only means by which NO biosynthesis and NO-dependent processes can be enhanced without utilization of synthetic NO donors such as nitroglycerin, and there is intense interest in the use of arginase inhibitors as possible therapeutic agents in the treatment of male and female sexual arousal disorders.[6−8]

In the first step of the arginase reaction, the metal-bridging hydroxide ion attacks the guanidinium carbon of L-arginine to form a tetrahedral intermediate, which subsequently collapses to form products ornithine and urea (Figure 1).[9]

To date, much inhibitor design effort has focused on the study of reactive boronic acid substrate analogues, in which the electrophilic boron atom is isosteric with the electrophilic guanidinium carbon of L-arginine.[7,10] The X-ray crystal structures of boronic acid inhibitor complexes with arginases I and II reveal that the trigonal planar boronic acid moiety undergoes nucleophilic attack to form the tetrahedral boronate anion, which in turn is isosteric with the tetrahedral intermediate and its flanking transition states in the arginase reaction (Figure 2a).[6−8]

To explore possible arginase inhibitors with preformed tetrahedral centers that mimic the catalytic tetrahedral intermediate (in contrast with the reactive tetrahedral center of a boronate anion), we now report the design, synthesis, and evaluation of L-amino acids bearing tetrahedral sulfonamide side chains. On the basis of analysis of arginase−boronate complexes,[6−8] the key structural requirement for inhibitor binding to arginase is a group that can simultaneously coordinate to manganese and donate a hydrogen bond to Asp-128. Since sulfonamide inhibitors bind to the zinc enzyme carbonic anhydrase in a comparable manner,[11] we hypothesized that a sulfonamide inhibitor would

* Corresponding author: Tel 215-898-5714; fax 215-573-2201.

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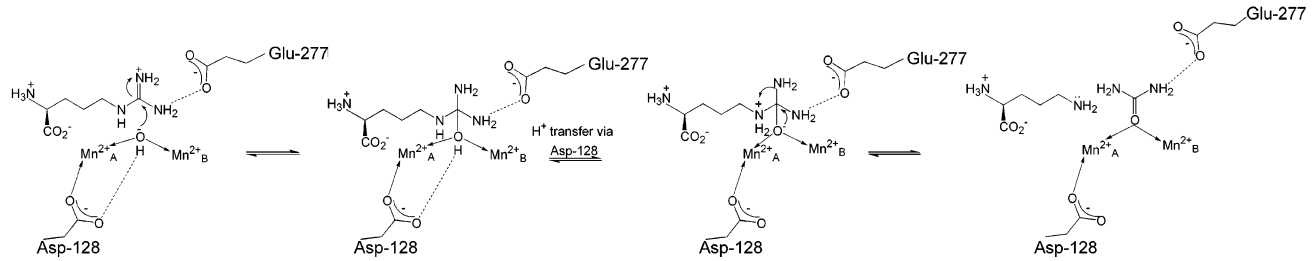

Figure 1. Arginase mechanism: hydrolysis of L-arginine occurs by nucleophilic attack of metal-bridging hydroxide ion at the guanidinium carbon of the substrate.

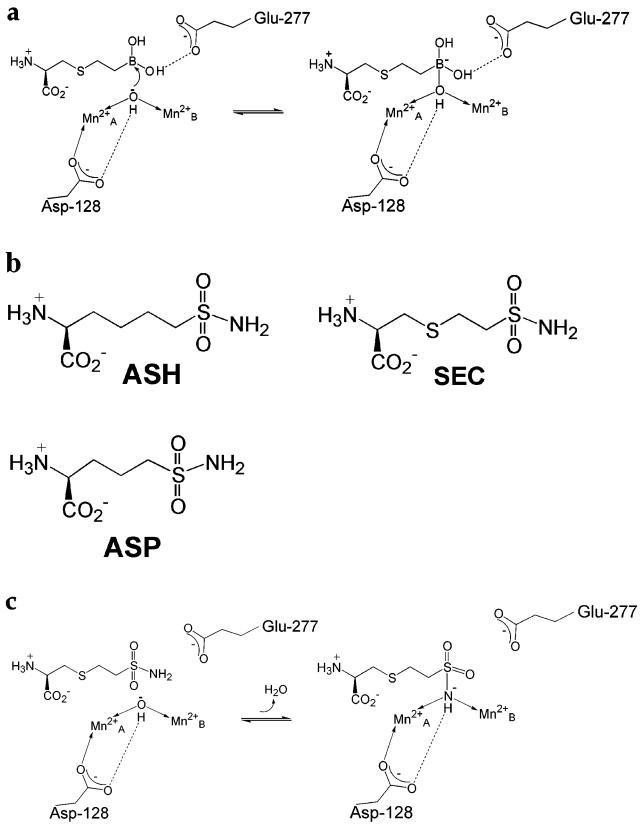

Figure 2. (a) The boronic acid analogue of L-arginine, *S*-(2-boronoethyl)-L-cysteine (BEC), undergoes nucleophilic attack by the metal-bridging hydroxide ion in the arginase active site. The resulting tetrahedral boronate anion mimics the tetrahedral intermediate (and its flanking transition states) in the hydrolysis of L-arginine (Figure 1). (b) Tetrahedral amino acid sulfonamides designed to inhibit arginase. These compounds contain preformed tetrahedral centers that mimic the tetrahedral intermediate and its flanking transition states in the arginase reaction (Figure 1). (c) Binding mode of SEC in the arginase active site.

behave similarly in the active site of arginase. Having measured millimolar levels of inhibition by various alkyl and aromatic sulfonamides against arginase (unpublished results), we proceeded with the study of *S*-(2-sulfonamidoethyl)-L-cysteine (SEC), (*S*)-2-amino-6-sulfonamidohexanoic acid (ASH), and (*S*)-2-amino-5-sulfonamidopentanoic acid (ASP) (Figure 2b).

This work culminates with the X-ray crystal structure determination of the arginase I−SEC complex, which reveals that the sulfonamide moiety of SEC symmetrically bridges the binuclear manganese cluster in the enzyme active site (Figure 2c).

Experimental Section

Synthesis of Amino Acid Sulfonamides. Except as otherwise indicated, reactions were carried out under a nitrogen atmosphere in flame- or oven-dried glassware, and solvents were freshly distilled. Diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone. Dichloromethane and triethylamine were distilled from calcium hydride. All other reagents and solvents were used without further purification from commercial sources. Organic extracts were dried over $MgSO_4$ or Na_2SO_4. Reactions were monitored by thin-layer chromatography (TLC) with 0.25-mm E. Merk precoated silica gel plates and visualized with ninhydrin solution (0.1% ninhydrin in 95% *n*-butanol, 4.5% water, and 0.5% glacial acetic acid). Flash column chromatography was carried out with E. Merk silica gel 60 (230−240 mesh ASTM). ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-500 spectrometer. Chemical shifts were expressed in parts per million (ppm) and referenced to $CDCl_3$, CD_3OD, or D_2O. The syntheses of (*S*)-2-amino-5-sulfonamidopentanoic acid (ASP) **7a** and (*S*)-2-amino-6-sulfonamidohexanoic acid (ASH) **7b** are as follows.

General Synthesis of (*S*)-1-*tert*-Butyl-bis[(2-*tert*-butoxycarbonyl)-amino]-6-bromohexanoate 3b. (*S*)-1-*tert*-Butyl-bis[(2-*tert*-butoxycarbonyl)amino]-6-hydroxyhexanoate **2b** (0.7 g, 1.73 mmol) and carbon tetrabromide (1.149 g, 3.47 mmol) were dissolved in dry THF (10 mL) and cooled to ∼15 °C. To this cooled solution was added Ph_3P (0.9 g, 3.47 mmol), and the mixture was stirred for 2 h. The solvent was removed on a rotary evaporator and the crude residue was chromatographed on silica gel (10% EtOAc in hexanes). Yield: 0.67 g (87%). ^1H NMR (500 MHz, $CDCl_3$): δ 4.71 (d, 1H), 3.42 (t, 2H), 2.07−2.04 (m, 1H), 1.94−1.86 (m, 3H), 1.51−1.46 (m, 2H), 1.51 (s, 18H), 1.45 (s, 9H).

(*S*)-1-*tert*-Butyl-bis[(2-*tert*-butoxycarbonyl)amino]-5-bromopentanoate 3a. The general procedure is the same as for the synthesis of **3b**. Yield: 89%. ^1H NMR ($CDCl_3$): δ 4.72 (dd, 1H), 3.47−3.38 (m, 2H), 2.21−2.18 (m, 1H), 2.04−1.90 (m, 3H), 1.54 (s, 18H), 1.45 (s, 9H). Exact mass calcd for ($C_{19}H_{34}O_6NBr$ + Na) (ESI) 474.23; found 474.17; isotope 476.18.

General Synthesis of (*S*)-1-*tert*-Butyl-[(2-*tert*-butoxycarbonyl)-amino]-6-sulfonylhexylamide 6b. To a solution of sodium thiosulfate pentahydrate (0.80 g, 3.22 mmol) in water (20 mL) was added (*S*)-1-*tert*-butyl-bis[(2-*tert*-butoxycarbonyl)amino]-6-bromohexanoate **3b** (1.0 g, 2.15 mmol) in MeOH (20 mL), and the mixture was refluxed for 2.5 h. The solvent was then removed on a rotary evaporator. The resulting suspension was chilled to 10 °C in an ice bath and then crushed ice (5 g) and glacial acetic acid (5 mL) were added. While the mixture was being stirred vigorously, chlorine was passed as rapidly as possible for 30 min. The extract was washed with 5% sodium bisulfite solution until it became colorless and then dried over $MgSO_4$. The solvent was removed on a rotary evaporator. A suspension of sulfonyl chloride **5b** in anhydrous ether (20 mL) was stirred at room temperature and treated with ammonia (gas) for 45 min. The crude product, with one N-Boc group deprotected, was purified by silica gel chromatography. Yield: 0.378 g, three steps overall 48%. ^{1}NMR ($CDCl_3$): δ 5.08 (s, 1H, NH-

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Boc), 4.71 (s, 2H, NH_2-SO_2), 4.20 (s, 1H), 3.12 (t, 2H), 2.05−1.78 (m, 4H), 1.70−1.52 (m, 2H), 1.51 (s, 9H), 1.44 (s, 9H). Exact mass calcd for ($C_{15}H_{30}N_2O_6S$ + Na) (ESI) 389.26; found 389.50.

(*S*)-1-*tert*-Butyl-[(2-*tert*-butoxycarbonyl)amino]-5-sulfonylpentylamide 6a. The general procedure is the same as for the synthesis of **6b**. Yield: 42%. ^{1}NMR ($CDCl_3$): δ 5.17 (s, 1H, NH-Boc), 4.93 (s, 2H, NH_2-SO_2), 4.18 (s, 1H), 3.12−3.30 (m, 2H), 1.90−1.99 (m, 3H), 1.75−1.79 (m, 1H), 1.51 (s, 9H), 1.44 (s, 9H). Exact mass calcd for ($C_{14}H_{28}N_2O_6S$ + Na) (ESI) 375.25; found 375.46. Anal. Calcd for $C_{14}H_{28}N_2O_6S$: C, 47.72; H, 7.95; N, 7.95. Found: C, 47.54; H, 8.26; N, 7.55.

General Synthesis of (*S*)-2-Amino-6-sulfonamidohexanoic acid 7b. To a −78 °C chilled, stirred solution of **6b** (100 mg, 0.27 mmol) in CH_2Cl_2 (5 mL) was slowly added BCl_3 (1 M, 0.54 mL, 0.54 mmol). The reaction mixture was stirred for 5 min at −78 °C and then a flow of nitrogen gas was allowed to pass over the mixture to evaporate the solvent. The crude product was purified by recrystallization with acetone/acetonitrile. In an alternative purification method, the mixture was concentrated under reduced pressure and the crude product was purified on a Dowex 50W-X8 ion-exchange resin (H^+ form). The column was washed with water and then with 0.5 M ammonia solution. Yield: 35 mg (52%). ^{1}H NMR (CD_3OD): δ 3.84 (s, 1H), 3.11 (t, 2H), 2.12−1.82 (m, 4H), 1.69−1.51 (m, 2H). Exact mass calcd for ($C_6H_{14}O_4N_2S$ + H) (ESI) 211.14; found 211.10.

(*S*)-2-Amino-5-sulfonamidopentanoic Acid 7a. The general procedure is the same as for the synthesis of **7b**. Yield: 38%. ^{1}H NMR (D_2O): δ 3.89 (t, 1H), 3.29 (t, 2H), 2.11−1.81 (m, 4H). Exact mass calcd for ($C_5H_{12}O_4N_2S$ + H) (ESI) 197.14; found 197.06.

Kinetic Assays. Arginase inhibition by ASH was evaluated by a modified version of the fixed-point radioactive assay developed by Rüegg and Russell.[12] Assay mixtures contained 100 mM 2-(cyclohexylamino)ethanesulfonic acid (CHES)-NaOH (pH 9.0), 100 μM $MnCl_2$, 0.05 μCi of [*guanidino*-^{14}C]-L-arginine, 10 mM unlabeled L-arginine for arginase I (10 × K_M) and 24 mM L-arginine for arginase II, and varying concentrations of ASH in a 50 μL volume per centrifuge tube. Reactions were started by the addition of 5 μL of a 0.14 μg enzyme solution and incubated for 5 min. Reactions were quenched by the addition of 400 μL of "stop" solution [0.25 mM acetic acid (pH 4.5), 7 M urea, 10 mM unlabeled L-arginine, and a 1:1 (v/v) slurry of Dowex W-X8 in water]. Each reaction mixture was vortexed immediately after the addition of "stop" solution, gently mixed for an additional 10 min, and centrifuged at 6000 rpm for 10 min. A 200 μL volume of the supernatant was removed and 3 mL of scintillation liquid (EcoScint) was added in preparation for liquid scintillation counting in a Beckman counter (model SL 1801). For data analysis, the plot of v_0/v as a function of inhibitor concentration was expected to be linear for a simple competitive inhibitor, and the slope of the line was used to calculate K_i using the equation for competitive binding (v_0 and v were the observed velocities measured in the absence and presence of inhibitor, respectively).

Surface Plasmon Resonance. The binding affinity of SEC to arginase I was determined by surface plasmon resonance.[13a,b] All experiments were carried out on a BIAcore 3000 instrument. Briefly, arginase I was immobilized via the primary amine groups onto a carboxymethylated dextran matrix in two flow cells of a CM5 biosensor chip assembly (Biacore, Inc.). After immobilization, the remaining carboxyl groups on the matrix were suppressed with ethanolamine. The highest density of active arginase I was achieved at pH 5. The two remaining flow cells in the flow cell block were not derivatized. One served as a reference surface to correct for bulk refractive index changes, while the other served as a control to ensure that SEC did not bind nonspecifically to the dextran matrix. Samples containing either *S*-(2-boronoethyl)-L-cysteine (BEC)[7] (used as control for positive binding) or SEC were randomized and injected in triplicates across the four flow cells. Stock solutions for inhibitors were prepared in 50 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (Hepes) (pH 7.5), 100 μM $MnCl_2$, and 150 mM KCl, which was also used as the running buffer. Biosensor data were collected at 25 °C for triplicate injections of a series of six concentrations of SEC (800−157 μM) and BEC (30−0.463 μM). A flow rate of 100 μL/min was used to minimize mass transport limitations. No binding was observed for BEC or SEC in the absence of immobilized arginase I, demonstrating that nonspecific binding did not occur. Data for inhibitor binding were fit to the equation describing a 1:1 Langmuir interaction model,[14] where R is the concentration of the molecular complex formed, C_A is the concentration of ligand A, and R_{max} is the total concentration of the immobilized entity:

$$dR/dt = k_a C_A(R_{max} - R) - k_d R$$

This fit yielded the association rate (k_a), the dissociation rate (k_d), and the dissociation constant ($K_d = k_d/k_a$).

Crystallography. Recombinant rat arginase I was expressed in *Escherichia coli* and purified by modification of previously described procedures.[15] Specifically, cultures were grown at 37 °C in LB medium containing 30 μg/mL kanamycin until $A_{600} = 0.8$ and then were induced with 0.2 mg/mL isopropyl thiogalactoside (IPTG). After harvesting, cells were resuspended in 50 mM Hepes-KOH (pH 7.5), 1 mM phenylmethanesulfonyl fluoride (PMSF), 1 mM ethylenediaminetetraacetic acid (EDTA), and a final concentration of 1 mg/mL lysozyme. The cells were sonicated and $MnCl_2$ was added to a final concentration of 20 mM. The supernatant was placed in a 60 °C water bath for 30 min and subsequently centrifuged at 20000*g* for 30 min. The supernatant was dialyzed exhaustively in 50 mM Hepes-KOH (pH 7.5), centrifuged at 20000*g* for 30 min, applied to a Sigma Reactive Red column, and eluted with a 0.0−0.3 M linear gradient of KCl in 50 mM Hepes-KOH (pH 7.5). The protein was concentrated to 12−16 mg/mL by centrifugation at 5000*g* at 4 °C in Amicon Ultra concentrators. The concentration of arginase was determined by measuring the absorbance at 280 nm using an extinction coefficient of 1.09 mL mg^{-1} cm^{-1}.

For cocrystallization of the enzyme−inhibitor complex, *S*-(2-sulfonamidoethyl)-l-cysteine (SEC) was purchased from Qventas (Newark, DE). Crystals of the arginase−SEC complex were prepared at 4 °C by equilibrating a hanging drop containing 3−5 μL of protein solution [12−16 mg/mL arginase I, 50 mM *N*,*N*-bis(2-hydroxyethyl)-glycine (bicine; pH 8.5), 5 mM $MnCl_2$, and 5 mM SEC] and 3−5 μL of precipitant solution [20% poly(ethylene glycol) 8000, 50 mM bicine (pH 8.1), and 5 mM $MnCl_2$] against 1 mL of precipitant solution in the well reservoir. Crystals appeared after 4 weeks. Crystals belonged to space group $P3_2$ with unit cell parameters $a = b = 88.9$ Å and $c = 112.5$ Å. Data collection statistics are recorded in Table 1.

Diffraction data were collected from a single flash-cooled crystal of the arginase I−SEC complex at the Cornell High Energy Synchrotron Source (CHESS), beamline F-1. Intensity data integration and reduction were performed with the HKL suite of programs.[16] Initial phases were determined by molecular replacement with the program AmoRe,[17] using

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Table 1. Data Collection and Refinement Statistics

resolution (Å)	2.8
total reflections (*N*)	204 073
unique reflections (*N*)	23 105
completeness (%) (last shell)	92.4 (82.5)[a]
R_{merge} (last shell)[b]	0.098 (0.287)[a]
reflections used in refinement (test set)	19037 (2099)[a]
R_{cryst}[c]	0.251
R_{free}[c]	0.290
rms deviations	
bonds (Å)	0.008
angles (deg)	1.4
dihedral angles (deg)	23.4
improper dihedral angles (deg)	1.1

[a] Numbers in parentheses refer to the outer 0.1 Å shell of data. [b] R_{merge} for replicate reflections, $R = \Sigma|I_h - \langle I_h\rangle|/\Sigma\langle I_h\rangle$; I_h = intensity measured for reflection *h*; $\langle I_h\rangle$ = average intensity for reflection *h* calculated from replicate data. [c] Crystallographic *R* factor, $R_{cryst} = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ for reflections contained in the working set. Free *R* factor, $R_{free} = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ for reflections contained in the test set held aside during refinement (5% of total). $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively.

the trimer of native rat liver arginase I as a search model.[9] Iterative rounds of refinement and rebuilding of the native model were performed with the programs CNS[18] and O,[19] respectively. The inhibitor SEC was built into the unbiased electron density map in the final stages of refinement. Strict noncrystallographic symmetry was employed at the beginning of the refinement and later relaxed to appropriately weighted constraints as judged by R_{free}. Refinement statistics are reported in Table 1. Atomic coordinates of the arginase I−SEC complex have been deposited into the Protein Data Bank[20] (http://www.rcsb.org/pdb) under accession code 1R1O. Figures were generated with BOBSCRIPT[21] and Raster3D.[22]

Results and Discussion

Amino Acid Sulfonamides. The synthesis of (*S*)-2-amino-6-sulfonamidohexanoic acid (ASH) **7b** utilized the commercially available L-glutamic acid derivative (*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid, from which the olefin (*S*)-1-*tert*-butyl-2-bis[(2-*tert*-butoxycarbonyl)amino]-5-hexenoate **1b** was prepared according to published procedures.[23] Hydroboration of olefin **1b** with a diborane−THF complex in THF and purification by silica gel column chromatography afforded alcohol **2b** in 78% yield, subsequent bromination of which provided **3b** in 87% yield. Compound **4b** was prepared from the reaction of (*S*)-bromide **3b** with sodium thiosulfate salt in MeOH/H_2O (1:1) at reflux temperature.[24] Subsequent chlorination of sodium alkylthiosulfate **4b** in dilute acetic acid solution followed by treatments with ammonia gas in dry ethyl ether afforded the desired sulfonamide derivative **6b** with an overall 48% yield for the three steps (**3b** to **6b**).[25,26] Complete deprotection[10] with BCl_3 yielded ASH, **7b**, a white solid in 52% yield. The synthesis of the shorter amino acid sulfonamide, (*S*)-2-amino-5-sulfamidopentanoic acid (ASP) **7a**, was achieved by a synthetic strategy identical to that utilized for the synthesis of ASH except that (*S*)-1-*tert*-butyl-bis[(2-*tert*-butoxycarbonyl)-amino]pentandioate ester **1a** was used as a starting material, reduction of which with $NaBH_4$ in *i*-PrOH/H_2O (4:1) provided the alcohol **2a** in 64% yield. We note that a previously reported synthesis[27] of ASH yields a racemic mixture, whereas this procedure allows for the simple and efficient synthesis of a variety of chiral amino acid sulfonamide derivatives by use of commercially available chiral amino acids as starting materials.

Scheme 1

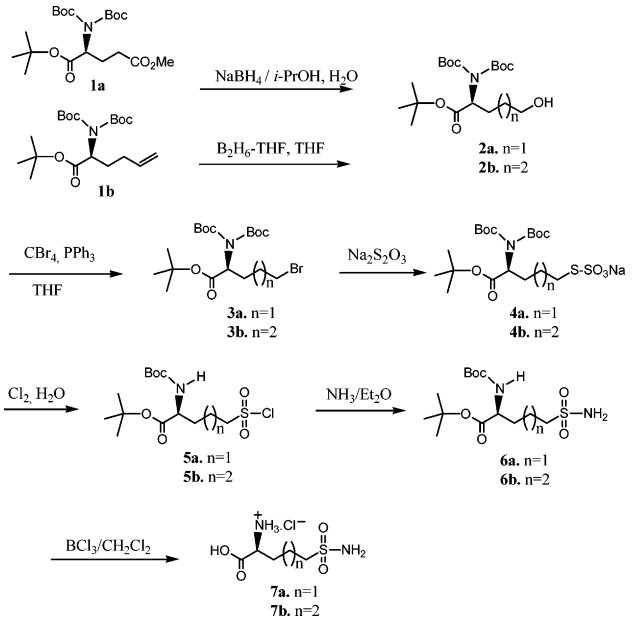

Table 2: Arginase−Inhibitor Binding Affinities

	kinetic assay K_i (μM)		surface plasmon resonance K_d (μM)	
compounds[a]	arginase I	arginase II	arginase I	arginase II
BEC	0.4−0.6[b]	0.23[c]	2.56[d]	
SEC			52.0	
ASH	90	67		
ASP	500			

[a] Structures shown in Figure 2. [b] See ref 7. [c] See ref 8. [d] The arginase I−BEC interaction measured by isothermal titration calorimetry yields K_d = 2.22 μM, consistent with the results of surface plasmon resonance (see ref 7).

Arginase-Inhibitor Binding Affinities. The surface plasmon resonance technique has been used successfully in measuring the binding of inhibitors to carbonic anhydrase II.[13c−e] Therefore, given the success of this technique in studying protein−small molecule interactions, we used it to study arginase−SEC and arginase−BEC interactions. The inhibitor BEC was used as a positive control since its K_d was previously determined by kinetic methods and isothermal titration calorimetry.[7] Inhibition of arginase I and II by ASH and ASP was measured at pH 9.5 as described in the Experimental Section. In general, there is good agreement among the results of these different techniques (Table 2).

Among the three amino acid sulfonamide inhibitors studied, the lowest affinity was measured for ASP with K_i = 500 μM

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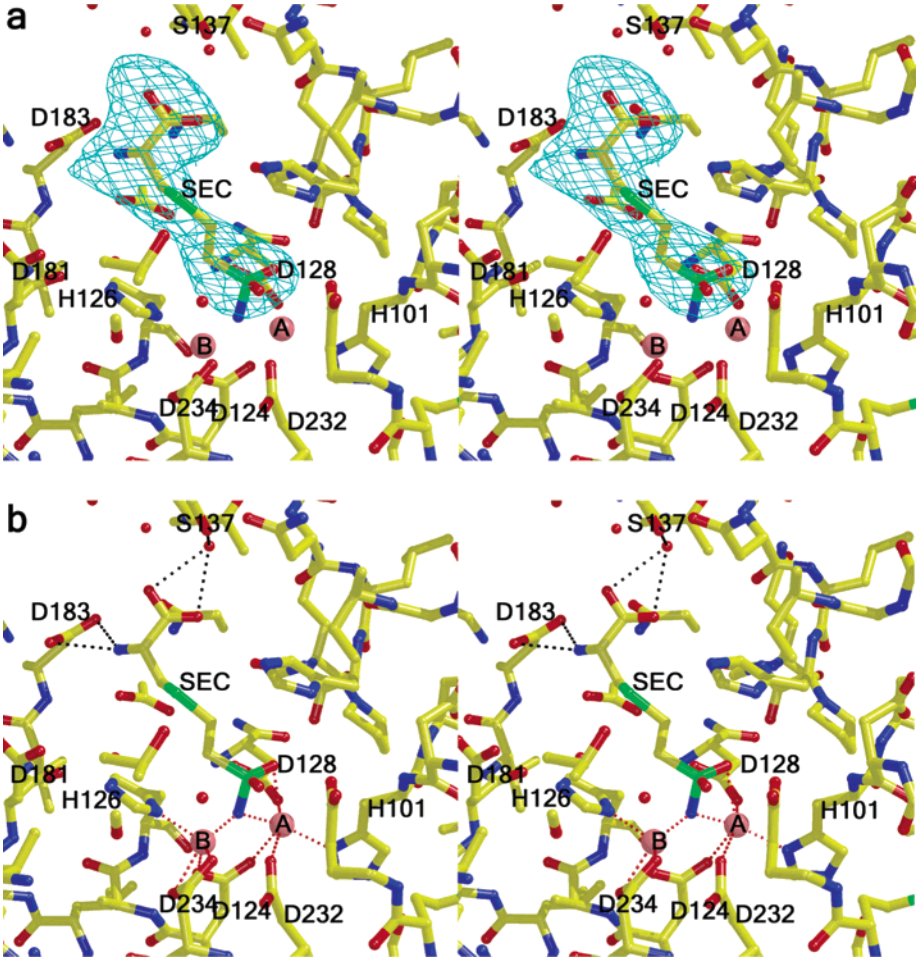

Figure 3. Arginase I−SEC complex. (a) Omit electron density map of SEC in the arginase active site. The map is contoured at 4.8σ, and selected active-site residues are indicated. Atoms are color-coded as follows: C = yellow, O = red, N = blue, S = dark green; manganese ions appear as pink spheres and water molecules appear as smaller red spheres. (b) Intermolecular interactions in the arginase I−SEC complex. Manganese coordination interactions are designated by red dashed lines, and hydrogen bonds are indicated by black dashed lines.

against arginase I. This was not too surprising, since ASP served as a control for weak binding due to its side chain being one methylene group shorter than that of L-arginine. The highest affinity was measured for SEC with K_d = 52.0 μM against arginase I. Accordingly, we focused our structural studies on the arginase I−SEC complex.

Structure of the Arginase I−SEC Complex. No major tertiary or quarternary structural changes are triggered by inhibitor binding to the enzyme active site, and the rms deviation of 308 C_α atoms is 0.346 Å between the arginase I−BEC[7] and arginase I−SEC complexes. The tetrahedral sulfonamide group of SEC binds to the binuclear manganese cluster and the ionized sulfonamide NH^- group symmetrically bridges Mn_A^{2+} and Mn_B^{2+} (Mn^{2+}−N separations = 2.0 Å) and donates a hydrogen bond to Asp-128 (Figures 2c and 3).

Of course, the sulfonamide NH^- group must be ionized in order to provide two electron pairs for simultaneous coordination to Mn_A^{2+} and Mn_B^{2+}. The Mn_A^{2+}−Mn_B^{2+} separation increases from 3.3 to 3.5 Å (comparable increases are observed upon the binding of other inhibitors to arginase I).[6,7,28] One sulfonamide oxygen coordinates to a formerly vacant site on Mn_A^{2+} (Mn_A^{2+}−O separation = 2.4 Å), while the second sulfonamide oxygen accepts a hydrogen bond from a solvent molecule, which in turn donates a hydrogen bond to Asp-234 and accepts a hydrogen bond from the backbone NH group of Thr-246.

The Mn_A^{2+} ion is coordinated by Asp-232 Oδ1, Asp-124 Oδ1, Asp-128 Oδ1, and His-101 Nδ1, in addition to the sulfonamide NH^- and O groups, with distorted octahedral geometry. The Mn_B^{2+} ion is coordinated by Asp-124 Oδ2, Asp-234 Oδ1, Asp-234 Oδ2, and His-126 Nδ1, in addition to the sulfonamide NH^- group, with distorted octahedral geometry. Asp-232 Oδ1 is 2.8 Å away from Mn_B^{2+}, which indicates an electrostatic interaction but is too far to be considered an inner-sphere coordination interaction.

The α-amino group of SEC donates hydrogen bonds to both carboxylate oxygens of Asp-183 (Figure 3). The α-carboxylate oxygens of SEC accept a bifurcated hydrogen bond from a solvent molecule. In turn, this solvent molecule hydrogen bonds with the hydroxyl group of Ser-137. The binding mode of the α-amino and α-carboxylate groups differs from the binding of these same groups in the arginase I−BEC complex.[7] Specifically, the α-carboxylate group of SEC loses direct hydrogen-bond contacts with Ser-137 and Asn-130 and solvent-mediated hydrogen bonds with Asn-139 and Thr-135. The α-amino group retains direct hydrogen-bond contact with Asp-183 but loses solvent-mediated hydrogen bonds with Asp-181, Glu-186, and

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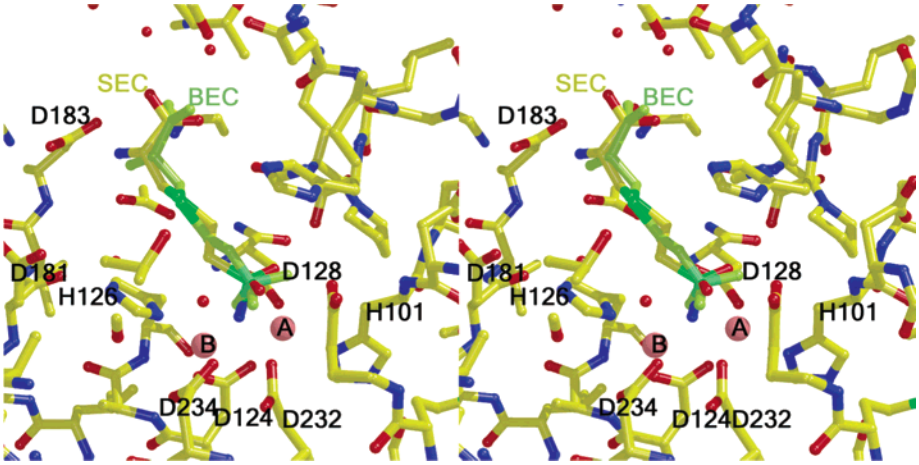

Figure 4. Superposition of the arginase I−SEC complex and the binding conformation of BEC in the arginase I−BEC complex (pale green).

the backbone carbonyl group of Gly-142. Possibly, the corresponding solvent molecules are not observed due to the modest 2.8 Å resolution of the structure determination. Alternatively, differences in the interactions of α-substituents might arise from other differences in the binding of the inhibitors. For example, the position of the sulfonamide NH^- group of SEC extends 0.5 Å more deeply into the active site compared with the corresponding boronate hydroxyl group of BEC; additionally, the $S\gamma-C\delta$ bond of SEC adopts an eclipsed conformation, whereas the $S\gamma-C\delta$ bond of BEC adopts a staggered conformation (Figure 4).

Interestingly, the binding of aliphatic sulfonamides to carbonic anhydrase reveals an inverse correlation between pK_a and pK_i.[29] Since inhibitory activity increases with increased functional group acidity in a system where the ionized sulfonamide displaces a metal-bound hydroxide ion, a sulfonamide group with lower pK_a likewise might be expected to inhibit arginase more strongly. However, in contrast with carbonic anhydrase inhibition, arginase inhibition by benzenesulfonamide (pK_a = 10.1, K_i = 13 mM) and 2,3,4,5,6-pentafluorobenzenesulfonamide (pK_a = 8.8, K_i = 4.1 mM) reveals at best only a weak increase of inhibitory activity with increased sulfonamide acidity (data not shown). Despite this contrast, structural features of sulfonamide binding to carbonic anhydrase and arginase I are quite similar: the ionized sulfonamide NH^- group coordinates to the active-site metal ion(s) in each enzyme with optimal N−metal ion separations of 1.9−2.1 Å, and the sulfonamide NH^- group simultaneously donates a hydrogen bond to an active-site residue (Thr-199 in carbonic anhydrase, Asp-128 in arginase I) with N−O separations of 2.7−3.1 Å. These structural features indicate strong sulfonamide−metal ion coordination interactions in each enzyme.

The pK_a values reported for alkyl sulfonamide chains comparable in length to the SEC, ASH, and ASP compounds, such as 1-butanesulfonamide, 1-hexanesulfonamide, and 1-octanesulfonamide, are on the order of 10.6−11.[30] In comparison, the pK_a values of alkyl boronic acids,[31] which reflect the hydration of the trigonal planar boronic acid to form the tetrahedral boronate anion [$R-B(OH)_3 \leftrightarrows R-B^-(OH)_4 + H^+$], are on the order of 10.3−10.5; the pK_a of the boronic acid side chain of BEC is reported to be 9.3 by Colleluori and Ash.[32] Accordingly, we speculate that slight differences in the acid−base chemistry of the metal-bridging sulfonamide or boronate group may at least partially contribute to the affinity differences between corresponding pairs of sulfonamide-based and boronic acid-based inhibitors such as SEC and BEC (Table 2).

Conclusions

Together with previous studies of sulfonamide inhibitors of carbonic anhydrase,[11] the results of the present work demonstrate that the sulfonamide group is an excellent ligand for the displacement of a hydroxide ion bound to either a single metal ion or a binuclear metal cluster. The sulfonamide group coordinates to one or two metal ions through an ionized NH^- group; possibly, the metal-bound hydroxide ion of the native metalloenzyme plays a role in deprotonating the sulfonamide NH_2 group in order to generate a more easily displaced metal-bound water molecule. In the arginase active site, the sulfonamide group of SEC symmetrically bridges the binuclear manganese cluster. This binding mode mimics the tetrahedral intermediate (and its flanking transition states) in the arginase reaction. Given the structural features evident in the arginase I−SEC complex, we conclude that the sulfonamide group may be successfully incorporated into inhibitors of other binuclear metalloenzymes.

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