Synthesis of (2*S*)-2-amino-7,8-epoxyoctanoic acid and structure of its metal-bridging complex with human arginase I†

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The synthesis of (2*S*)-2-amino-7,8-epoxyoctanoic acid is reported along with the X-ray crystal structure of its complex with human arginase I, revealing unique coordination interactions with two manganese ions in the enzyme active site.

Epoxides (also known as oxiranes) have been used successfully as irreversible inhibitors of a large variety of proteolytic enzymes, *e.g.*, cysteine proteases.[1] Although the oxirane moiety is stable in neutral aqueous solution and is only weakly electrophilic,[2] it becomes much more reactive upon interaction with a Lewis acid such as a metal ion or a hydrogen bond donor. Lewis acid activation facilitates oxirane reactions with strong nucleophiles such as activated serine and cysteine residues, and also with weaker nucleophiles such as the carboxylate groups of aspartate and glutamate residues.[3] Such is the catalytic strategy employed by mammalian epoxide hydrolases, in which the substrate oxirane moiety is activated by hydrogen bonds with two tyrosine residues for nucleophilic attack by an aspartate side chain.[4]

Although activation of the oxirane moiety is achieved by protonation in most enzymes, activation by metal coordination is occasionally observed. For example, the oxirane moiety of fosfomycin is activated by coordination of the epoxide to the single Mn^{2+} in the active site of the fosfomycin resistance protein FosA.[5] Additionally, carboxypeptidase A is inhibited by 2-benzyl-3,4-epoxybutanoic acid through a mechanism involving oxirane–Zn^{2+} coordination prior to nucleophilic attack by a glutamate residue.[6]

Despite the significant potential of the oxirane moiety in the design of ligands and inhibitors of metalloenzymes, very little structural information is available regarding oxirane–metal coordination. Only one crystal structure of an intact epoxide coordinated to a metal ion is available in the Protein Data Bank (PDB), that of the fosfomycin–Mn^{2+} interaction in the active site of FosA.[5] Here, we report the synthesis of (2*S*)-2-amino-7,8-epoxyoctanoic acid (**3**) and the X-ray crystal structure of its complex with human arginase I. This manganese metalloenzyme catalyzes the hydrolysis of L-arginine to form L-ornithine and urea and is overexpressed in various desease states such as asthma[7] and atherosclerosis.[8] The structure of this complex reveals the first view of an intact oxirane bridging a binuclear manganese cluster.

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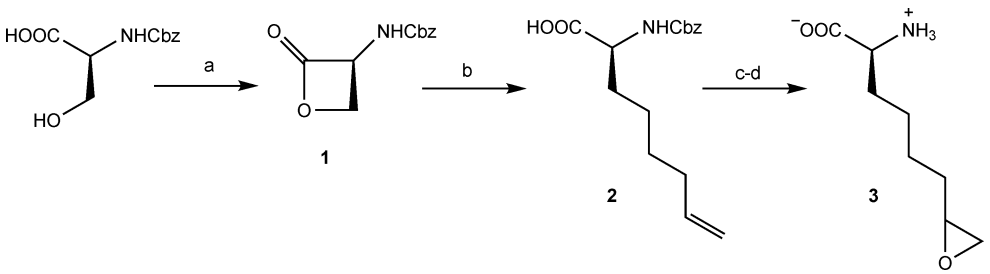
The synthesis of **3** is shown in Scheme 1.‡ Briefly, Cbz-protected (*S*)-2-amino-7-octenoic acid was prepared from the previously reported β-lactone (**1**)[9] according to the procedure developed by Vederas.[10] Compound **2** was then epoxidized with MCPBA to form a mixture of two diastereomers of Cbz-protected **3**. Deprotection of the amino group was achieved using a relatively mild hydrogenation procedure. Full experimental details for the synthesis and characterization of compounds **1–3** can be found in the ESI.† We note that the syntheses of protected versions of **2** and a derivative of **3** have been reported previously.[11,12]

For X-ray crystallography, human arginase I was overexpressed in *E. coli*, purified, and crystallized as described previously.[13] The structure of the arginase–**3** complex at 1.51 Å resolution was determined from crystals soaked with 40 mM solution of the inhibitor for 3 days. Initial phases for the electron density map were obtained by molecular replacement using the program Phaser[14] with chain A of the unliganded human arginase I complex (PDB accession code 2ZAV, less solvent molecules[13a]) used as a search probe against twinned data. In order to refine and calculate electron density maps, structure factors amplitudes ($|F_{obs}|$) derived from twinned data (I_{obs}) were deconvoluted into structure factor amplitudes corresponding to twin domains A and B ($|F_{obs/A}|$ and $|F_{obs/B}|$, respectively) as described.[13a] Iterative cycles of model building with the graphics program O[15] and refinement using torsion angle dynamics as implemented in CNS[16] improved the protein structure as monitored by R_{twin} and $R_{free/twin}$. Group B-factors were utilized during refinement. In the final stages of refinement the majority of water molecules were automatically fit into residual electron density peaks using a cutoff of 3.0σ, which improved the R_{twin} and $R_{free/twin}$ values. In the later stages of refinement a gradient omit map calculated with CNS[16] clearly showed the presence of a strong peak corresponding to **3** bound to the active sites of monomers A and B of the asymmetric unit. Disordered segments at the N- and C-termini (M1-T5 and N319-K322) were absent in the experimental electron density and are omitted from the final model. Data collection and refinement statistics are reported in Table 1.

The 7(*R*) diastereomer of **3** binds exclusively in the active site of human arginase I (Fig. 1a). The binding of **3** does not cause any significant conformational changes in the active site, and the r.m.s. deviation is 0.18 Å for 313 Cα atoms between the structures of the complexed and unliganded enzymes. However, an important structural change is observed in the manganese coordination polyhedron: the oxirane oxygen displaces the bridging hydroxide ion observed in the unliganded enzyme[13a] and bridges $Mn^{2+}{}_A$ and $Mn^{2+}{}_B$ with an average coordination distance of 2.3 Å. This distance is comparable to Mn^{2+}–O distances observed in protein[17] and small molecule[18] crystal structures.



Scheme 1 Synthesis of (2*S*)-2-amino-7,8-epoxyoctanoic acid (**3**).‡ *Reagents and conditions*: (a) PPh_3, DEAD, −78 °C; (b) 4-pentenylmagnesium bromide, CuBr·SMe_2, THF/SMe_2, −23 °C; (c) MCPBA, CH_2Cl_2, rt; (d) H_2, $Pd(OH)_2/C$, 25 min. DEAD = diethyl azodicarboxylate; MCPBA = 3-chloroperoxybenzoic acid.

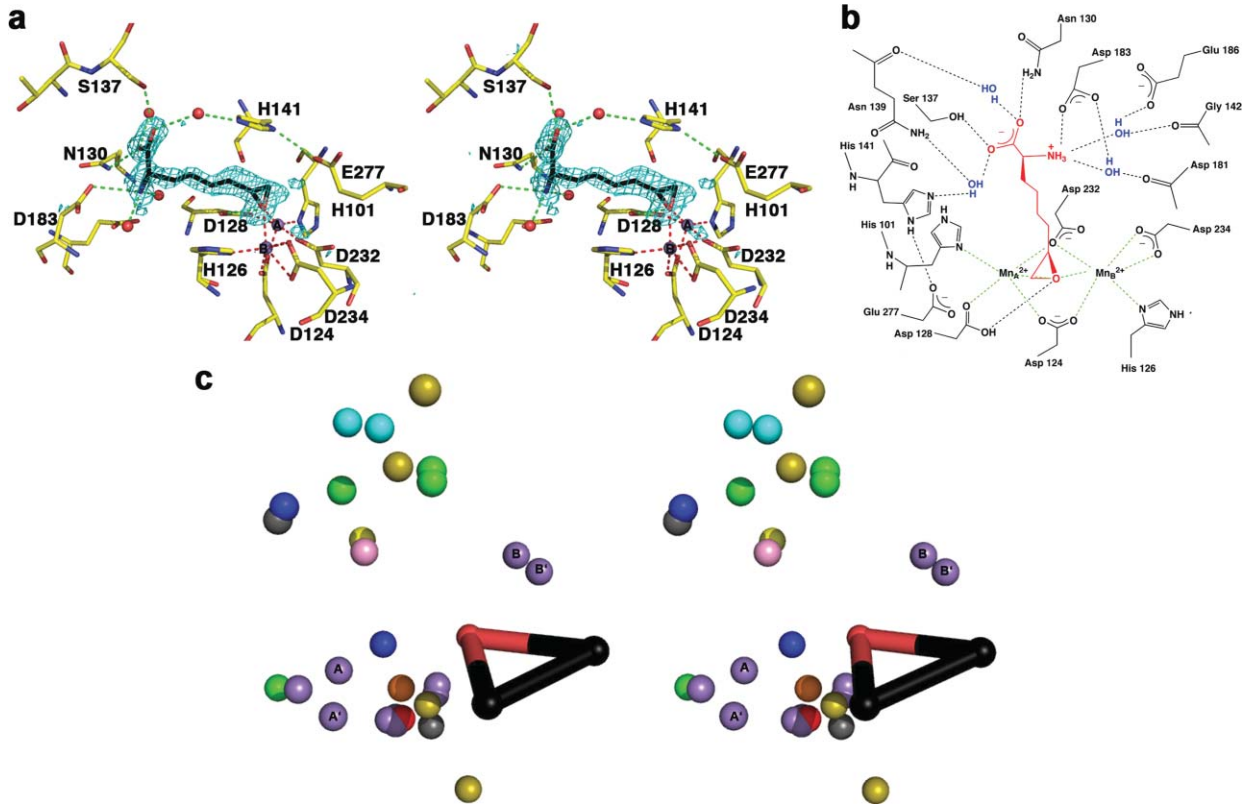


Fig. 1 (a) Stereoview of a simulated annealing gradient omit map showing **3** (3.2σ contour, cyan) bound in the active site of human arginase I (monomer A). Dashed lines indicate manganese coordination (red) and hydrogen bond (green) interactions. Atom color codes: carbon (yellow), oxygen (red), nitrogen (blue), manganese (violet). Carbon atoms of **3** are black. (b) Summary of intermolecular interactions for arginase I–**3** complex. (c) Oxirane–metal interactions retrieved from the CSD and PDB. Atom color codes: C (black), O (red), Mn (violet), Ga (olive), Li (green), Cd (blue), Hg (gray), Na (cyan), Ag (pink), Ca (yellow), Ru (orange) and Zr (red). The Mn^{2+} ions labelled A/B and A′/B′ represent the metal ion positions in monomers A and B, respectively, of the human arginase I–**3** complex.

As observed in the binding of other amino acid inhibitors to human arginase I,[13b] the α-carboxylate and α-amino groups of **3** are anchored to the active site of arginase by three direct and four water-mediated hydrogen bonds. Additionally, the oxirane oxygen receives a hydrogen bond from D128, which is presumably protonated to accommodate this interaction.

Analyses of the PDB and the Cambridge Structural Database (CSD) indicate that the human arginase I–**3** complex is only the second crystal structure ever determined of a metal-bridging oxirane, the first such complex being *rac*-(μ_2-3,4,5,6-tetrafluorobenzene-1,2-diyl)-(μ_2-2-methyloxirane-*O*,*O*)-bis(chloromercury) (CSD accession code MOCNAT).[19] Interestingly, the structure of a gallium–oxirane complex reveals Ga–O separations of 1.951 Å and 2.886 Å, the latter of which is too long to consider as an inner-sphere interaction (CSD accession code CABPIF).[20]

A full search for oxirane–metal complexes in the CSD was performed using the ConQuest program. A total of 14 different crystal structures were retrieved with metal–O separations of 1.75–3.50 Å.[21] Each of these structures was inspected visually to confirm the oxirane fragment and checked against the original literature

Table 1 Data collection and refinement statistics

	Human arginase I-**3** complex
Data collection	
Resolution/Å	50.0–1.51
Unique reflections measured	99149 (9976)
R_{merge} [a]	0.060 (0.485)[b]
$I/\sigma(I)$	12.7 (2.9)[b]
Completeness (%)	98.8 (99.5)[b]
Multiplicity	3.6 (3.6)[b]
Refinement	
Reflections used in refinement/test set	96593/4719
R_{twin}, $R_{twin/free}$ [c]	0.152 (0.252)[b], 0.193 (0.258)[b]
Protein atoms[d]	4762
Water molecules[d]	332
Atoms of **3**[d]	24
Manganese ions[d]	4
r.m.s. deviations	
Bond lengths/Å	0.006
Bond angles/°	1.4
Dihedral angles/°	22.7
Improper dihedral angles/°	1.0
Average B-factors/Å²	
Main chain	23
Side chain	25
Manganese ions	18
3	21
Solvent	30

[a] $R_{merge} = \sum|I - \langle I\rangle|/\sum I$, where I is the observed intensity and $\langle I\rangle$ is the average intensity calculated for replicate data. [b] Number in parentheses refer to the outer 0.1 Å shell of data. [c] $R_{twin} = \sum|[F_{calc/A}|^2 + |F_{calc/B}|^2]^{1/2} - F_{obs}|/\sum|F_{obs}|$ for reflections contained in the working set. $|F_{calc/A}|$ and $|F_{calc/B}|$ 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. The same expression describes $R_{twin/free}$, which was calculated for test set reflections excluded from refinement. [d] Per asymmetric unit. Data deposition: final coordinates and structure factors have been deposited in the Protein Data Bank: PDB entry code 3DJ8.

report of the structure determination. Scatterplots were obtained by superimposing the triangular oxirane group of each complex and allowing the metal ions to ride into position using the Insight II (ACCELRYS) program.

From the 14 small molecule structures reported in the CSD and 2 protein–oxirane structures reported in PDB (including the arginase I–**3** complex), a total of 29 unique oxirane–metal interactions with metal–O separations of 3.5 Å or less have been retrieved and are superimposed in Fig. 1c. As can be seen, metal ions involved in these complexes include Mn (11), Ga (4), Li (4), Cd (2), Hg (2), Na (2), Ag (1), Ca (1), Ru (1) and Zr (1). Metal ions tend to cluster in two regions above and below the oxirane plane, as a result of the interactions with the two lone electron pairs of the oxygen of the oxirane. The coordination geometry of the oxirane oxygen of **3** to $Mn^{2+}{}_B$ lies outside of the clusters evident in Fig. 1c, perhaps because the oxirane oxygen also interacts with D128 and thereby must optimize simultaneously interactions with two metal ions and a hydrogen bond donor.

Despite the apparent activation of the oxirane oxygen of **3** by bridging the two Mn^{2+} ions in the arginase active site, and despite the proximity of H141 and E277 to the electrophilic carbons of the oxirane moiety, a covalent bond between the enzyme and **3** does not form. Apparently, H141 and E277 are either too distant or too poorly oriented for nucleophilic attack. As a consequence, however, we have successfully captured a unique metalloprotein complex in which the oxirane oxygen simultaneously coordinates to two Mn^{2+} ions.

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Notes and references

‡ Characterization for **3**: ^{1}H NMR (360 MHz, D_2O) δ 1.39–1.95 (m, 8H), 2.73 (app t, $J = 3.8$ Hz, 1H), 2.97 (app t, $J = 4.3$ Hz, 1H), 3.16–3.21 (m, 1H), 3.75 (t, $J = 6.0$ Hz, 1H); ^{13}C NMR (D_2O) δ 24.06, 24.85, 30.51, 31.07, 48.24, 54.03, 54.81, 175.36; HRMS (ESI) m/z calcd. for $C_{16}H_{22}NO_5Na$ $(M + Na)^+$: 196.0950, found: 196.0954.

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