Iron superoxide dismutases in eukaryotic pathogens: new insights from Apicomplexa and *Trypanosoma* structures

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Received 26 November 2014

Accepted 27 February 2015

Edited by W. N. Hunter, University of Dundee, Scotland

Keywords: iron superoxide dismutase; *Trypanosoma*; Apicomplexa.

PDB references: iron superoxide dismutase from *L. major*, 4f2n; putative iron superoxide dismutase from *T. cruzi* bound to iron, 4h3e; superoxide dismutase from *B. bovis*, 4k2w; 4yet

Supporting information: this article has supporting information at journals.iucr.org/f

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Prior studies have highlighted the potential of superoxide dismutases as drug targets in eukaryotic pathogens. This report presents the structures of three iron-dependent superoxide dismutases (FeSODs) from *Trypanosoma cruzi*, *Leishmania major* and *Babesia bovis*. Comparison with existing structures from *Plasmodium* and other trypanosome isoforms shows a very conserved overall fold with subtle differences. In particular, structural data suggest that *B. bovis* FeSOD may display similar resistance to peroxynitrite-mediated inactivation via an intramolecular electron-transfer pathway as previously described in *T. cruzi* FeSOD isoform B, thus providing valuable information for structure-based drug design. Furthermore, lysine-acetylation results in *T. cruzi* indicate that acetylation occurs at a position close to that responsible for the regulation of acetylation-mediated activity in the human enzyme.

1. Introduction

Superoxide dismutases (SODs) are a ubiquitous family of key enzymes that protect aerobic organisms against toxic radicals by converting superoxide to oxygen and peroxide (McCord & Fridovich, 1969). Numerous structural studies since the early 1980s have revealed that these enzymes belong to three classes with distinct folds and reaction mechanisms: the Cu,ZnSODs, MnSOD/FeSODs and NiSODs (Ursby *et al.*, 1999; Perry *et al.*, 2010). As the first barrier against reactive oxygen species, iron-dependent superoxide dismutases (FeSODs) make attractive drug targets against parasitic protozoa, in particular trypanosomatids and malaria-causing parasites (Turrens, 2009). Multiple FeSOD genes exist in each genome: in Trypanosomatidae four FeSODs isozymes have been described with different subcellular localizations, based on experimental data in *Trypanosoma brucei* and phylogenetic analysis (Dufernez *et al.*, 2006; Wilkinson *et al.*, 2006), while in Apicomplexa two FeSOD isoforms, one cytosolic and one mitochondrial, have been described in *Plasmodium falciparum* (Boucher *et al.*, 2006) and *Babesia hylomysci* (Becuwe *et al.*, 1994).

The Seattle Structural Genomics Center for Infectious Disease (SSGCID) has partnered with TDR Targets to select potential drug targets in eukaryotic pathogens (see §2.1). The set of targets thus identified included 12 SODs from four species and yielded the three structures that are the subject of this study: the first FeSOD structures to be solved from

B. bovis (PDB entry 4yet, 1.75 Å resolution) and *L. major* (PDB entry 4f2n, 1.85 Å resolution), as well as the first released mitochondrial FeSODA structure from *T. cruzi* (PDB entry 4h3e, 2.25 Å resolution). Iron and manganese SODs are very closely related (Abreu & Cabelli, 2010); the assignment of these targets as FeSODs was based on the presence of a conserved sequence signature for FeSODs (NN/QAAQ) at position 116 in the alignment shown in Fig. 3, compared with F/NG/AGG for MnSODs (Bécuwe *et al.*, 1996). However, unambiguous assignment to the FeSODs remains to be experimentally established. Another structure of the same *T. cruzi* isoform A was released six months later together with the structure of a B isoform (Martinez *et al.*, 2014). Based on structural analysis and mass-spectrometric data, the authors proposed a mechanism to explain the resistance of cytosolic FeSODB to peroxynitrite inactivation, in contrast to mitochondrial FeSODA. Another study compared the structural features of FeSODs in parasites with human MnSOD to assess the druggability of the active site (Bachega *et al.*, 2009). The present study proposes the evaluation of the presence of a structural signature indicative of resistance to peroxynitrite inactivation and the comparison of the active site to that of human MnSOD, using the structures from *L. major*, a trypanosomatid from a related genus, and from *B. bovis*, a malaria-related parasite. In addition, we present the results of a lysine-acetylation study using the *T. cruzi* mitochondrial FeSODA to provide additional insight into the regulation of this essential enzyme.

2. Methods

2.1. Target selection

Genome-wide target prioritization was performed using the TDR Targets Database resource (<http://tdrtargets.org>; Agüero *et al.*, 2008), which houses complete genome information integrated with a number of functional and manually curated data sets for various tropical disease pathogens. A generic query for selecting desirable targets (Crowther *et al.*, 2010) was used to obtain the top 100 ranked genes from different species belonging to Apicomplexa, Kinetoplastida, helminths and mycobacteria. The complete query strategies along with the numerical weights used for ranking genes are published on the TDR targets 'Posted Lists' pages (<http://tdrtargets.org/published/browse/t/366>; <http://tdrtargets.org/published/browse/t/390>; <http://tdrtargets.org/published/browse/t/394>; <http://tdrtargets.org/published/browse/t/395>). Orthologs and paralogues of these prioritized target genes were then added using data from the OrthoMCL database (<http://orthomcl.org>; Chen *et al.*, 2006) for the purpose of providing multiple target options for every selected gene. Further filters were applied to the target sequences based on the presence of transmembrane domains, the size of the protein and the number of cysteine residues as previously described (Phan *et al.*, 2014). The final shortlisted genes were entered into the SSGCID structural genomics pipeline for cloning, expression, purification and crystallization studies. The three FeSOD proteins for which

structures are described in this work were included after being identified as orthologs (OrthoMCL group ID OG4_10139) of the prioritized targets.

2.2. Protein expression and purification

Iron superoxide dismutases from *L. major* strain Friedlin (mitochondrial *LmFeSODA*; EupathDB LmjF.08.0290; UniProt Q4QIE0), spanning the length of the mature protein from residues 22 to 230, and *B. bovis* strain T2Bo (*BbFeSOD*; EupathDB BBOV_IV011480; UniProt O15904), spanning the full-length protein from residues 1 to 199, were cloned into the ligation-independent cloning (LIC; Aslanidis & de Jong, 1990) expression vector pAVA0421 encoding a cleavable 6×His fusion tag followed by the human rhinovirus 3C protease cleavage sequence (MAHHHHHHMGTLEAQTQGPGR-ORF). Mitochondrial iron superoxide dismutase from *T. cruzi* strain CL Brener (*TcFeSODA* with native mutation L128F; EupathDB Tc00.1047053509775.40; UniProt Q4DCQ3), spanning the full-length protein from residues 1 to 233, was cloned into the expression vector BG1861, which expresses the protein product fused to a minimal noncleavable 6×His tag (MAHHHHHH) at the N-terminus.

Each construct encoding the FeSOD from a parasite was transformed into chemically competent *Escherichia coli* BL21 (DE3) Rosetta cells. An overnight culture was grown in LB broth at 37°C and was used to inoculate 2 l ZYP-5052 auto-induction medium, which was prepared as described by Studier (2005). FeSOD was expressed in a LEX bioreactor in the presence of ampicillin (50 µg ml⁻¹). After 24 h at 25°C the temperature was reduced to 15°C for a further 60 h. The sample was centrifuged at 4000g for 20 min at 4°C and the cell paste was flash-frozen in liquid nitrogen and stored at -80°C.

During the purification process, the frozen cell pellet was thawed and completely resuspended in lysis buffer {20 mM HEPES pH 7.4, 300 mM NaCl, 5% glycerol, 30 mM imidazole, 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 10 mM MgCl₂, 3 mM β-mercaptoethanol, 1.3 µg ml⁻¹ protease-inhibitor cocktail, 0.05 mg ml⁻¹ lysozyme}. The resuspended cell pellet was then disrupted on ice for 15 min with a Branson Digital 450D Sonifier (70% amplitude with alternating cycles of 5 s pulse on and 10 s pulse off). The cell debris was incubated with 20 µl Benzonase nuclease at room temperature for 40 min. The lysate was clarified by centrifugation with a Sorvall RC5 at 10 000 rev min⁻¹ for 60 min at 4°C in an F14S rotor (Thermo Fisher) and the supernatant was syringe-filtered through a 0.45 µm cellulose acetate filter (Corning Life Sciences, Lowell, Massachusetts, USA). The lysate was purified by IMAC using a HisTrap FF 5 ml column (GE Biosciences, Piscataway, New Jersey, USA) equilibrated with binding buffer [20 mM HEPES pH 7.0, 300 mM NaCl, 5% glycerol, 30 mM imidazole, 1 mM tris(2-carboxyethyl)phosphine (TCEP)] and eluted with 500 mM imidazole in the same buffer. The eluted FeSOD was concentrated and further resolved by size-exclusion chromatography (SEC) using a Superdex 75 26/60 column (GE Biosciences) equilibrated in SEC buffer (20 mM HEPES pH

Table 1

Crystallographic data-collection and refinement statistics.

Values in parentheses are for the highest of 20 resolution shells.

	<i>L. major</i> FeSODA	<i>B. bovis</i> FeSOD (SAD)	<i>T. cruzi</i> FeSODA
Data collection			
Space group	<i>P</i> 1	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 2 ₁ 2 ₁ 2 ₁
Unit-cell parameters			
<i>a</i> (Å)	47.03	55.75	47.65
<i>b</i> (Å)	102.74	69.09	75.68
<i>c</i> (Å)	168.24	11481	117.45
α (°)	91.66	90.0	90.0
β (°)	96.36	90.0	90.0
γ (°)	95.01	90.0	90.0
Wavelength (Å)	0.976484	1.54	1.54
Resolution range (Å)	19.77–1.85 (1.90–1.85)	50.00–1.75 (1.80–1.75)	50.0–2.25 (2.31–2.25)
Unique reflections	256038	44891	20226
Completeness (%)	96.2 (91.5)	98.5 (92.4)	97.0 (94.4)
<i>R</i> _{merge} [†]	0.046 (0.418)	0.101 (0.372)	0.137 (0.37)
Mean <i>I</i> / σ (<i>I</i>)	(2.80)	10.92 (2.97)	(5.99)
Refinement			
Resolution range (Å)	19.77–1.85 (1.90–1.85)	50.00–1.75 (1.80–1.75)	44.19–2.25 (2.31–2.25)
<i>R</i> _{cryst}	0.173 (0.263)	0.143 (0.198)	0.171 (0.170)
<i>R</i> _{free} [‡]	0.203 (0.289)	0.176 (0.245)	0.212 (0.266)
R.m.s.d., bonds (Å)	0.01	0.008	0.012
R.m.s.d., angles (°)	1.283	1.084	1.407
Total No. of atoms	21460	3628	3700
Wilson <i>B</i> factor (Å ²)	31.63	24.71	19.81
Average <i>B</i> factor (Å ²)			
All atoms	28.83	17.00	13.31
Water	13.35	29.87	18.40
Metal ions	19.35	10.25	10.37
Residues in favored regions (%)	98.1	98.8	97.9
Residues in disallowed regions (%)	0.0	0.0	0.0
<i>MolProbity</i> score [percentile]	1.1 [98th]	1.44 [99th]	1.1 [99th]
PDB code	4f2n	4yet	4h3e

[†] $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$. [‡] $R_{\text{free}} = \sum_{hkl} |F_{\text{obs}}| - |F_{\text{calc}}| / \sum_{hkl} |F_{\text{obs}}|$. The free *R* factor was calculated using 5% of the reflections, which were omitted from the refinement.

7.0, 300 mM NaCl, 5% glycerol, 1 mM TCEP) attached to an ÄKTA FPLC system (GE Biosciences). Peak fractions were collected and pooled based on purity-profile assessment by SDS–PAGE. Concentrated pure protein was flash-frozen in liquid nitrogen and stored at -80°C . The final protein concentrations (*Bb*FeSOD, 62.8 mg ml⁻¹; *Lm*FeSODA, 35.3 mg ml⁻¹; *Tc*FeSODA, 34.4 mg ml⁻¹) were determined by UV spectrophotometry at 280 nm using a Nanodrop spectrophotometer and were calculated using extinction coefficients (*Bb*FeSOD, 61 420; *Lm*FeSODA, 47 900; *Tc*FeSODA, 44 920) obtained with *ProtParam* (Gasteiger *et al.*, 2005). Final purity (>95%) was assayed by SDS–PAGE electrophoresis.

2.3. Crystallization

The *T. cruzi*, *L. major* and *B. bovis* orthologs of FeSOD were all produced as part of the standard protein-production pipeline of the SSGCID and therefore were all prepared for crystallization in a standard buffer: 25 mM HEPES pH 7.0, 500 mM sodium chloride, 2 mM DTT, 0.025% sodium azide, 5% glycerol. The three proteins were crystallized using identical standardized methods (sitting-drop vapour diffusion in 96-well plates with drops composed of 400 nl protein solution and 400 nl crystallization solution equilibrated against a reservoir containing 80 μ l crystallization solution). The only differences in the methods used to crystallize the samples were

the concentration of the proteins and the crystallization solution used to obtain crystals. *Tc*FeSODA was crystallized at a concentration of 23.05 mg ml⁻¹ with a precipitant composed of 0.1 M HEPES pH 7.0, 30% Jeffamine ED-2001. *Lm*FeSODA was crystallized at 35.33 mg ml⁻¹ with a precipitant composed of 18.4% PEG 3350, 184 mM potassium formate. The *B. bovis* ortholog of FeSOD was crystallized at 62.8 mg ml⁻¹ from a precipitant solution consisting of 0.1 M Tris–HCl pH 8.5, 25% PEG 3350.

Crystals were prepared for data collection by transfer into a cryoprotectant solution consisting of the crystallization solution plus 25%(v/v) ethylene glycol and then were flash-cooled by plunging a crystallization mounting loop directly into liquid nitrogen.

2.4. Data collection and structure determination

X-ray diffraction data for *Lm*FeSODA were collected using an ADSC Quantum 315 CCD detector on beamline 5.0.3 at the Advanced Light Source in Berkeley, California, USA. Diffraction data for the *B. bovis* and *T. cruzi* FeSOD structures were collected using a Rigaku Saturn 944+ CCD detector on a Rigaku FR-E+ SuperBright rotating-anode generator. All data sets were reduced using *XDS* (Kabsch, 2010) and scaled using *XSCALE* (Kabsch, 2010).

All structures could be solved by molecular-replacement methods with *Phaser* (McCoy *et al.*, 2007) as implemented in the *CCP4* software suite (Winn *et al.*, 2011) using a previously published set of MnSOD/FeSOD structure coordinates as a search model: PDB entry 1isa for *LmFeSODA* (42% identity;

Lah *et al.*, 1995), PDB entry 2a03 for *BbFeSOD* (65% identity; Structural Genomics of Pathogenic Protozoa Consortium, unpublished work) and PDB entry 4f2n (the *LmFeSODA* structure) for *TcFeSODA*. The data for *BbFeSOD* were of sufficient quality to enable phasing by single anomalous dispersion (SAD) using the *PHENIX* software suite (Grosse-Kunstleve & Adams, 2003). During the SAD phasing process, the metal ions present in the structure were initially attributed as zinc, since the strength of the anomalous map peaks for the metals was similar to the signal observed for S atoms in the protein structure and the anomalous scattering factors for zinc and sulfur are very similar at the wavelength at which the data were collected (1.54 Å). However, interatomic distance measurements between the metal ions and the protein were more consistent with the expected values for iron rather than zinc ions (Harding, 2006). On this basis, the metal ion was modeled as iron and *PHENIX* was allowed to refine the occupancy at less than 100%, but further work is needed to establish the nature of the bound metal unambiguously. The models were subjected to manual rebuilding and inspection using *Coot* (Emsley *et al.*, 2010) and were refined using either *REFMAC5* (Winn *et al.*, 2001) for *LmFeSODA* and *TcFeSODA* or *phenix.refine* (Afonine *et al.*, 2012) for *BbFeSOD*. Data-collection and refinement statistics are given in Table 1.

2.5. Parasite cultures, mitochondria isolation and MS analysis

T. cruzi CL Brener strain epimastigotes were cultivated in liver infusion tryptose (LIT) medium containing 10% fetal bovine serum (FBS) at 28°C. Exponentially cultured parasites were used to isolate mitochondria using the well established protocol for *T. brucei* mitochondrial isolation as described in Panigrahi *et al.* (2009). Isolated mitochondrial fractions were lysed in 1× PBS and 1% Triton X-100 in the presence of protease inhibitors. To avoid protein deacetylation during sample preparation, the lysine deacetylase inhibitors

Figure 1

The FeSOD global fold is highly conserved: SSGCID structures of FeSOD from *B. bovis* (tan), FeSODA from *L. major* (blue) and FeSODA from *T. cruzi* (pink) aligned against the existing structures of FeSODB from *T. cruzi* (green) and *P. falciparum* (red). All figures in this manuscript were prepared with *UCSF Chimera* (Pettersen *et al.*, 2004).

Figure 2

Targeting protozoan FeSOD: the conserved lysine capping the active site is not present in the human enzyme. The active-site cavity of human *versus* protozoa SOD structures is shown colored by electrostatic potential. Left, taken from Bacheaga *et al.* (2009). Right, targets solved by SSGCID. Solvent-excluded surfaces were calculated using the *MSMS* package (Sanner *et al.*, 1996).

nicotinamide and butyric acid were added to the lysis buffers at a concentration of 20 mM each.

Protein samples were precipitated with acetone and dissolved in 8 M urea followed by reduction and alkylation. The peptides were cleaned using ZipTips Micro-C₁₈ (Millipore Corporation) before subsequent MS analysis. LC-MS/MS analysis was performed with an Easy-nLC 1000 (Thermo Scientific) coupled to an Orbitrap Elite mass spectrometer (Thermo Scientific). The LC system was configured in a vented format (Licklider *et al.*, 2002) and consisted of a fused-silica nanospray needle (PicoTip emitter, 50 µm internal diameter; New Objective) packed in-house with Magic C18 AQ 100 Å reverse-phase medium (Michrom Bioresources Inc.; 25 cm) and a trap (IntegraFrit Capillary, 100 µm internal diameter; New Objective) containing Magic C18 AQ 200 Å (2 cm). The peptide sample was diluted in 10 µl 2% acetonitrile and 0.1% formic acid in water, and 8 µl was loaded onto the column and separated using a two-mobile-phase system consisting of 0.1% formic acid in water (A) and 0.1% acetic acid in acetonitrile (B). A 90 or 180 min gradient from 7 to 35% acetonitrile in 0.1% formic acid at a flow rate of 400 nl min⁻¹ was used for chromatographic separations. The mass spectrometer was operated in a data-dependent MS/MS mode over the *m/z* range 400–1800. The mass resolution was set to 120 000. For

each cycle, the 15 most abundant ions from the scan were selected for MS/MS analysis using 35% normalized collision energy. Selected ions were dynamically excluded for 30 s. MS spectra were evaluated using Mascot v.2.5 (Matrix Science). Peptides were matched to *T. cruzi* CL Brener proteins (El-Sayed *et al.*, 2005). Enzyme specificity was set to trypsin/P, with up to two missed cleavages permitted. A peptide mass tolerance of ±10 p.p.m. and an MS/MS tolerance of ±0.5 Da was used. Carbamidomethyl cysteine was specified as a fixed modification, with oxidized methionine and acetylation of lysine residues allowed. The results were further analyzed with the Scaffold software (Proteome Software), which utilizes a Bayesian approach. High-scoring peptide identifications with protein probability ≥90 and peptide probability ≥90 were considered in the analysis.

3. Results and discussion

3.1. Structures of FeSODs from *T. cruzi*, *L. major* and *B. bovis*

The structures of FeSODs from *T. cruzi*, *L. major* and *B. bovis* were solved. Comparison with existing structures from *Plasmodium* and other trypanosome isoforms shows that

Figure 3

Repair of TcFeSODB inactivation through intramolecular electron transfer (IET) from the exposed cysteine Cys83 to Tyr35 (Martinez *et al.*, 2014) may be conserved in BbFeSOD. Bottom, multiple sequence structure-based alignment (Meng *et al.*, 2006) of the five protozoan structures in Fig. 1 showing the location of the residues involved in the IET pathway; top left, BbFeSOD (tan) superimposed on TcFeSODB (green) showing the conserved IET residues and surface-exposed BbFeSOD cysteine; top right, the same region in LmFeSODA and TcFeSODA showing residues at equivalent positions and the buried cysteine.

the overall fold is remarkably conserved, as has been reported previously (Bachega *et al.*, 2009; Fig. 1). This study described the presence in protozoans of a conserved lysine (Lys38 in *T. cruzi* FeSODB) capping the active site that is not conserved in the human enzyme. A comparison of the active-site cavity confirms the prediction from sequence analysis that the relative position of this lysine is conserved in *T. cruzi*, *L. major* and *B. bovis* (Fig. 2). However, the lysine side chain appears to be flexible and no hydrogen bond to the amine group could be detected in any of the three structures, which would limit the extent of obstruction of the active site. For instance, compared with the *T. cruzi* FeSODB structure, the conserved lysine N $^{\epsilon}$ atom in isoform A is 5 Å further away from the catalytic iron and the side chain does not appear to cover the cavity. The functional significance of a conserved lysine at this position in protozoans remains undefined.

Another intriguing question regarding this family of proteins in these pathogenic protozoans concerns the potential functional difference between isoforms. In particular, an intramolecular electron-transfer (IET) pathway was proposed to explain the resistance to peroxynitrite-mediated inactivation of *T. cruzi* cytosolic FeSOD isoform B (*TcFeSODB*) compared with the mitochondrial isoform A (Martinez *et al.*, 2014). The mechanism for inactivation involved Cys83 acting as an electron donor to repair the nitration of the universally conserved Tyr35. According to this model, the pathway should be absent from mitochondrial *L. major* FeSODA. In *B. bovis*, the FeSOD isoforms have not been annotated and the cellular localization is unknown: assuming that at least one isoform exists in the cytosol and the mitochondria, the presence or absence of the IET pathway could indicate which isoform was solved. A structure-based sequence alignment and mapping of

the residues involved in the IET pathway in *TcFeSODB* onto the three SSGCID structures shows that the cysteine residue closest to *TcFeSODB* Cys83 is buried in the *Leishmania* structure and likewise in the *T. cruzi* isoform A, but is exposed in *B. bovis* (Fig. 3). Moreover, the methionine involved in the IET pathway in *TcFeSODB* (Met82) is conserved in *B. bovis* and not in the *L. major* and *T. cruzi* SODAs, where it is replaced by an isoleucine. This suggests that the *BbFeSOD* structure is from a cytosolic isoform and that it may display similar resistance to peroxynitrite-mediated inactivation as *TcFeSODB*.

3.2. The active site of *TcFeSODA* and acetylation

Protein acetylation has emerged as an important post-translational modification, and several recent proteome studies from different organisms, including protozoan parasites, have identified more than 3000 potential acetylation substrates localized in different cellular compartments (Kim *et al.*, 2006; Choudhary *et al.*, 2009; Jeffers & Sullivan, 2012; Miao *et al.*, 2013). The addition of an acetyl group to a protein can change the size and charge of the affected amino acids as well as the local hydrophobicity, each of which can affect the activities of the protein. Using high-throughput proteome analysis from *T. cruzi* mitochondrial extracts, we were able to identify lysine-acetylated (K-ac) residues at positions Lys44 and Lys97 in *TcFeSODA* (Moretti *et al.*, in preparation). Acetylation has also been detected in human MnSOD and has been demonstrated to be important in the regulation of its enzymatic activity (Chen *et al.*, 2011). Of the four K-ac residues identified in human MnSOD, Lys68, Lys122, Lys130 and Lys221, only Lys68 was observed to be important in the

Figure 4

The lysine-acetylation site in *TcFeSODA*, Lys97, highlighted in blue (right), is located in the same region as the lysine-acetylation site Lys68 involved in activity regulation in human MnSOD (left).

regulation of SOD activity. A comparison of the *TcFeSODA* and human MnSOD structures shows that the relative position of the acetylated Lys97 residue in *T. cruzi* is similar to that of the Lys68 residue in human MnSOD (Fig. 4), suggesting that acetylation could regulate *TcFeSODA* activity using a mechanism comparable to that of the human enzyme. Residue Lys44, although conserved in trypanosomatids, is located on the surface and away from both the active site and the Lys97 acetylation site, which would indicate that it might not be important for regulation of *TcFeSODA* activity. Functional studies are under way to confirm whether acetylation at position Lys97 is important for regulation of *TcFeSODA* activity.

4. Conclusion

Despite sharing a remarkably conserved global fold, the SSGCID FeSOD structures presented here provide additional insights into this family of proteins in two classes of pathogenic protozoans; in particular, the structural data indicate that (i) the active site is capped by a conserved lysine in protozoa but not in humans, (ii) the IET pathway conferring resistance to peroxynitrite-mediated inactivation may be conserved in the *B. bovis* enzyme but not in mitochondrial isoforms from trypanosomatids and (iii) the *TcFeSODA* Lys97 acetylation site is found in a similar location to the lysine that regulates human MnSOD activity *via* acetylation.

Acknowledgements

The authors wish to thank the whole SSGCID team. This project was funded in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services under Contract No. HHSN272201200025C. NSM and SS were funded by Fundação de Amparo à Pesquisa do Estado de São Paulo–FAPESP: grants 2012/09403-8 and 2013/20074-9 to NSM and 2011/51973-3 to SS. KS was funded by National Institutes of Health Grant R01 AI078962. IC was supported by an American Heart Association fellowship (14POST18970046).

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