Enzymatic Ros-Scavenging System Is Regulated by Calcium in Trypanosomatids Via Tryparedoxin Peroxidases

Morais, M.A.B.¹; Souza, T.A.C.B.¹; Giuseppe, P.O.¹; Honorato, R.V.¹; Alegria, T.G.P.²; Oliveira, P.S.L.¹; Oliveira, M.A.²; Netto, L.E.S.²; Murakami, M.T.¹

¹Laboratório Nacional de Biociências, CNPEM, Campinas, SP, Brazil ²Dep. de Biologia, USP, São Paulo, Brazil

INTRODUCTION: Tryparedoxin peroxidase (TXNPx) is a peroxiredoxin essential for the main enzymatic scavenger system for reactive oxygen species (ROS) in trypanosomatids. TXNPx is crucial for survival during oxidative stress, enhances infectivity and alters susceptibility to drugs. The catalytic cycle includes an oxidation-reduction process and shift in enzyme quaternary structure (dimers and doughnut oligomers). The overall goal of this work is to shed light on the molecular mechanisms involved in the catalytic and oligomeric cycles in TXNPx.

MATERIAL AND METHODS: Mitochondrial TXNPx of Leishmania braziliensis (mLbTXNPx) was cloned and overexpressed in prokaryotic system, purified and crystallized. The crystal structure was solved by molecular replacement. Size-exclusion chromatography, dynamic light scattering and SAXS were used to study the oligomerization of LbTXNPx. Peroxidase assays, site-directed mutagenesis and molecular dynamics simulations were performed.

RESULTS AND DISCUSSION: This work describes the first crystallographic structure of a mitochondrial peroxiredoxin-1 from trypanosomatids in both dimeric and decameric forms, revealing different redox states of peroxidase cysteine and a putative intermediate state. DLS, aSEC and SAXS analyses indicate that protein decamers upon calcium binding, which is not observed for other Prxs. Site-directed mutagenesis and MD studies demonstrated that calcium promotes the decameterization by interacting with D108 from both molecules. Activity assays showed that calcium binding enhances the peroxidase activity in comparison to the dimeric form.

CONCLUSION: The profile of multiple redox states in our structures and the discovery of new regulatory mechanism of oligomerization by calcium provided a new molecular perspective regarding the catalytic and oligomeric cycles in TXNPxs.

Keywords: Peroxiredoxin, structure, oligomerization.
Acknowledgements: FAPESP and CAPES.