MS06 Structural Enzymology

MS6-04

X-ray induced reduction of heme metal centres is protein-independent – implications for structural studies of redox sensitive proteins

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Abstract

X-ray crystallography is one of the main resources to obtain information on the coordination of molecules within the active site of metalloproteins on an atomistic level. Based on ligand coordination, interatomic distances and relative positioning of catalytic amino acids enzymologists try to understand the underlying electronic reaction mechanism. Therefore the exact redox status and conformation of the cofactor in question is of utmost importance. Unfortunately the redox active nature of metal cofactors makes them especially susceptible to irradiation induced photoreduction, making structural information obtained by photo-reducing X-ray sources the least trustworthy [1,2]. Here we present a study of the pre-steady state reduction kinetics of X-ray induced photo-reduction of six different model heme proteins to identify a reasonable dose-limit for the collection of non-reduced datasets for redox-active metallo enzymes. Using online-UV-vis spectroscopy we examined the reduction kinetics of the heme cofactor to understand the impact of sample-derived variables (protein, crystallization conditions, crystal morphology and cofactor) and irradiation-derived variables (dose and dose rate). We can show that the reduction kinetics solely depend on the dose, irrespective of the sample-derived variables (Figure 1) and define a protein-independent dose-limit of ~40 kGy, which corresponds to a 50% reduction. Furthermore, using standard macromolecular crystallography tools, we were able to collect and solve time-resolved low dose structures. We present structures of a model heme protein (KpDyP) in different defined redox states obtained by serial crystallography and dose-specific data splitting. These structures show photoreduction induced rearrangements in water coordination and conformation of the catalytically relevant residue Asp 143 [3] (Figure 2). The observed effects of photo-reduction highlight that care has to be taken when in-solution data of ferric proteins are rationalized by structural constraints derived from crystal structures of reduced enzymes [4]. Finally, we will present data for a pristine unreduced XFEL structure of KpDyP in its biologically relevant iron (III) oxidation state.

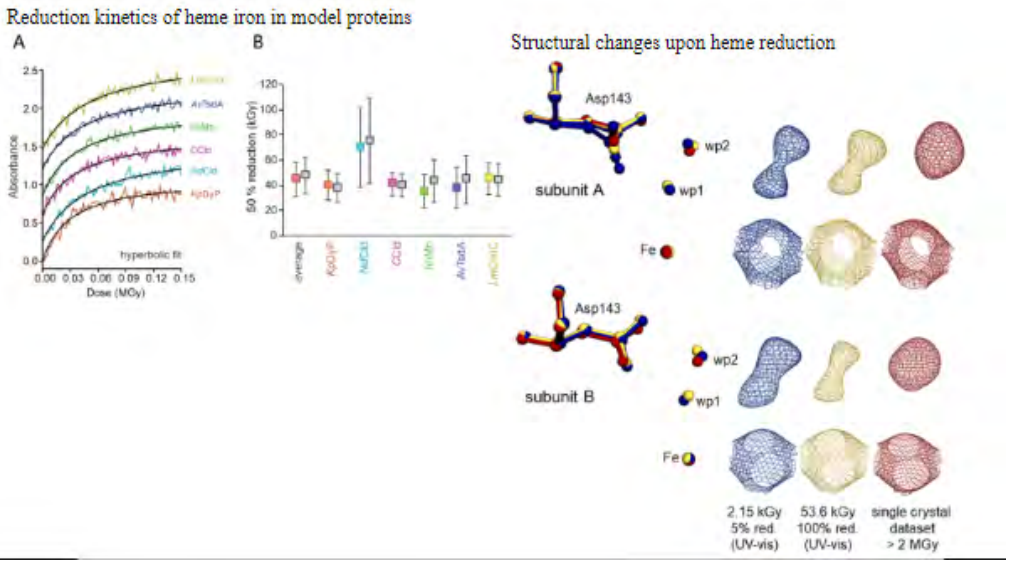
References

[1] G. I. Berglund, G. H. Carlsson, A. T. Smith, H. Szöke, A. Henriksen, J. Hajdu, Nature 417 (2002), 463-468

[2] T. Beitlich, K. Kühnel, C. Schulze-Briese, R. L. Shoeman, I. Schlichting, J Synchrotron Radiat 14 (2007), 11-23

[3] V. Pfanzagl, K. Nys, M. Bellei, H. Michlits, G. Mlynek, G. Battistuzzi, K. Djinovič-Carugo, S. Van Doorslaer, P.G. Furtmüller, C. Obinger, J. Biol Chem 293 (2018), 14823-14838

[4] V. Pfanzagl, J.H. Beale, H. Michlits, D. Schmidt, T. Gabler, C. Obinger, K. Djinovic-Carugo, S.Hofbauer, J. Biol Chem 295 (2020), 13488-13501


Reduction kinetics of heme iron in model proteins
A
B
Absorbance
Dose (MGy)
hyperbolic fit
50 % reduction (kGy)
average
Structural changes upon heme reduction
Asp143
subunit A
wp2
wp1
Fe
Asp143
subunit B
wp2
wp1
Fe
2.15 kGy
5% red.
(UV-vis)
53.6 kGy
100% red.
(UV-vis)
single crystal
dataset
> 2 MGy