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


1University of Natural Resources and Life Sciences, Department of Chemistry - Wien (Austria), 2Paul Scherrer Institute - Villigen (Switzerland), 3University of Vienna, Max F. Perutz Laboratories - Wien (Autriche), 4Paul Scherrer Institute - Villigen (S Switzerland uss)

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

Reduction kinetics of heme iron in model proteins

Structural changes upon heme reduction

Asp143

subunit A

wp0

wp1

Asp143

subunit B

wp2

wp3

Fe

2.15 kGy

(5% red)

(5% red)

53 kGy

100% red

angle crystal
data set

> 2 MGy