MS03 O1

Structural basis of the iron storage and delivery functions of frataxin S. Al-Karadaghi[1], T. Karlberg[1], U. Schagerlof[1], O. Gakh[2], S. Park[2], U. Ryde[3], M. Lindahl[1], K. Leath[4],E.Garman[4], G. Isaya[2].*[1,3]-Departments of Molecular Biophysics & Theoretical Chemistry, Lund University, Sweden;[2]-Departments of Pediatric & Adolescent Medicine and Biochemistry & Molecular Biology, Mayo Clinic College of Medicine, Rochester, USA;[4]-Department of Biochemistry, University of Oxford, UK.*
E-mail: salam.al-karadaghi@mbfys.lu.se

Keywords: catalytic mechanisms, iron storage, iron delivery

Frataxin performs key functions in iron delivery and detoxification via a currently unknown mechanism. The crystal structure of the iron-free and iron-loaded frataxin trimer and a single particle electron microscopic reconstruction of a 24-subunit iron-free and iron-loaded oligomers provide a basis for understanding the mechanisms of frataxin self-assembly in oligomeric particles, iron acquisition, detoxification and storage. They also provide an insight into the interplay between frataxin and other proteins, to which iron is delivered. The structure of the trimer suggests that a gated mechanism controls iron delivery to different targets and iron storage, a combination of critical biological roles not found in other known iron-binding proteins. Since the trimer seems to exhibit structural details consistent with both such roles, we propose that it represents the primary functional unit of frataxin. Moreover, the trimer structure suggests that mutations found in patients with Friedreich's ataxia may destabilize trimer formation. Thus, compounds that would stabilize the frataxin trimer may provide a means to maximize the frataxin function in individuals affected by Friedreich's ataxia. An additional remarkable feature of frataxin oligomers to be discussed in this presentation is the striking functional similarities of frataxin particles to the evolutionary unrelated ferritin superfamily of iron storage proteins.

MS03 O2

Insights into sucrose isomerization from Sucrose Isomerase crystal structures Nushin Aghajari, *CNRS/University Lyon 1, Lyon, France*
E-mail: n.aghajari@ibcp.fr

Keywords: Sucrose isomerase, specificity, enzyme-substrate complex

Trehalulose (α-D-glucosylpyranosyl-1,1-D-fructofuranose) and isomaltulose (α-D-glucosylpyranosyl-1,6-D-fructofuranose) are structural isomers of sucrose (α-D-glucosylpyranosyl-1,2-β-D-fructofuranoside) which have similar taste profiles and very similar physical and organoleptic properties to sucrose. Moreover they are noncariogenic and their absorption reduces the rate with which monosaccharides and insulin are released into the bloodstream, why they can be applied in diabetic and sports foods and drinks. Considering also that no side effects have been yet reported, these compounds could be ideal sucrose substitutes. Furthermore, the reducing property of isomaltulose makes it an attractive industrial precursor for the manufacturing of biosurfactants and biocompatible polymers.
We have recently cloned, purified, crystallized [1], [2] and solved the structures of the sucrose isomerases, MutB, from *Pseudomonas mesoacidophila* MX-45, which mainly produces trehalulose, and of SmuA from *Protaminobacter rubrum* which mainly produces isomaltulose. Since sucrose is an inexpensive and readily available D-glucose donor, the industrial potential of these enzymes for synthesis of trehalulose and/or isomaltulose is large. For optimal utilization of the enzymes, targeting controlled synthesis of these functional isomers starting from sucrose, it is necessary to minimize the side reactions. This requires a thorough analysis of substrate binding modes and of the specificity-determining sites in the 3D-structures.
We have investigated the catalytic mechanism and the specificities of MutB by combining mutagenesis and structural approaches, and report the first structure of a trehalulose synthase, and the first enzyme-substrate complex structures for a sucrose isomerase.

[1] Ravaud S.; Watzlawick H.; Mattes R.; Haser R.; Aghajari N. *Acta Cryst F* 2005, 61, 100.
[2] Ravaud S.; Watzlawick H.; Haser R.; Mattes R.; Aghajari N. *Acta Cryst F* 2006, 62, 74.

MS03 O3

Crystallographic and *in crystallo* Raman spectroscopic studies of iron-peroxide intermediates in superoxide reductase Gergely Katona[a], Philippe Carpentier[a], Vincent Nivière[c], Patricia Amara[a], Virgile Adam[b], Jeremy Ohana[a], Nikolay Tsanov[a], & Dominique Bourgeois[a,b] *[a]IBS, Institut de Biologie Structurale, Grenoble, France. [b]European Synchrotron Radiation Facility, Grenoble, France. [c]Laboratoire de Chimie et Biologie des Métaux, iRTSV-CEA/CNRS/Université J. Fourier, Grenoble, France.*
E-mail: gergely.katona@ibs.fr

Keywords: Raman spectroscopy, iron-peroxide intermediate, intermediate trapping

Iron-peroxide intermediates are involved in the catalytic cycle of many iron-containing enzymes. Such intermediates were also identified in superoxide reductase (SOR) a non-heme mononuclear iron-enzyme that neutralizes superoxide radicals [1-3]. By diffusing hydrogen peroxide into SOR crystals, we trapped iron(III)-(hydro)peroxo species. X-ray diffraction data and non-resonant Raman spectra recorded in crystallo revealed "end-on" iron-(hydro)peroxo configurations. Raman spectroscopy also monitored the influence of X-ray radiation on the trapped intermedate. The ^{18}O isotopic shifts of the iron-peroxide specific vibration bands confirmed the direct involvement of hydrogen peroxide in the formation of the intermediate.
The open SOR active site promotes the formation of transient hydrogen bond networks, which presumably assist the cleavage of the Fe-O bond in order to release the reaction product, hydrogen peroxide. Multiple observations of the SOR active sites in the asymmetric unit allowed us to propose a structural mechanism which directs the processing of reactive oxygen species without producing highly reactive iron (IV) oxo intermediates.