MS97.30.3

Acta Cryst. (2005). A**61**, C123

The Activation Process of *D.desulfuricans* ATCC 27774 [NiFe] Hydrogenase

Pedro M. Matias, *ITQB-UNL, Oeiras, Portugal.* E-mail: matias@itqb.unl.pt

In recent years, we have determined the 3-D structure of [NiFe] hydrogenase from the sulphate- and nitrate-respiring bacteria *D.desulfuricans* ATCC 27774. The active site of this enzyme, which catalyses the reversible reaction $H_2 \leftrightarrow 2H^+ + e^-$ is constituted of a Ni Fe heteronuclear diatomic metal core bonded to the protein chain by four cysteine residues, two of which bridge the metal atoms. The Fe atom is further coordinated by two CO and one CN ligands.

In the simplest description, three states are usually considered for the active site: unready, ready and active. These states have been characterised by EPR spectroscopy for several hydrogenases from different organisms.

Our crystallographic studies allowed us to obtain structural details of the active site in each one of the three states. A key result that emerged from this study was evidence for the coupling between Cys 536, Glu 24 (a highly conserved residue in [NiFe] hydrogenases from *Desulfovibrio* and related organisms) in proton transport off the active site. Cys 536 may even be implicated in the activation of the H-H bond prior to its heterolytic cleavage.

These results have in turn led to a proposed mechanism for the activation process of this enzyme, supported by DFT calculations.

Keywords: hydrogenase, active-site structure, activity and mechanism of enzymes

MS97.30.4

Acta Cryst. (2005). A**61**, C123

Time- and Position-resolved X-ray Scattering of Bone and Cartilage

Peter Fratzl[a], Himadri S. Gupta[a], Wolfgang Wagermaier[a], Paul Roschger[b], Aurélien Gourrier[a], Oskar Paris[a], [a]*Max Planck Institute of Colloids and Interfaces, Department of Biomaterials, Potsdam, Germany*. [b]*Ludwig Boltzmann Institute of Osteology, Wien, Austria*. E-mail: fratzl@mpikg.mpg.de

Most biological tissues including bone and cartilage are hierarchically structured and dynamically remodelled, and as a consequence, are heterogeneous in space and time. For a better understanding of the mechanical properties of these tissues, as well as for the characterization of bone diseases, it is essential to cover many length scales in structural investigation. X-ray diffraction and/or small angle scattering can be used to study the orientation and size of mineral particles as well as the spacing and orientation of collagen fibrils. When the specimen is scanned across a narrow X-ray beam, the micron and the nanometer scales are covered simultaneously, by the scanning procedure and the analysis of diffraction patterns, respectively. We have used this scanning technology to characterize individual trabeculae or osteons in intact macroscopic bone sections, as well as the bone cartilage interface and the dentin-enamel junction. One of the great advantages of the scanning diffraction approach is that the same specimens can be used for additional characterisation with other imaging techniques, such as electron, infrared or Raman imaging, as well as nanoindentation. Complementary to the scanning approach, *in-situ* methods utilize the high brilliance of synchrotron radiation to carry out time-resolved measurements at the fibrillar and molecular level to study deformation mechanisms in bone and biomineralized tissues.

Keywords: biomineralization, microbeam analysis, SAXS

MS97.30.5

Acta Cryst. (2005). A**61**, C123

A Mesoporous Pattern Created by Nature in Siliceous Spicules from Marine Sponges

Gianluca Croce[a], Marco Milanesio[a], DavideViterbo[a], Heinz Amenitsch[b], [a]*DISTA, Università del Piemonte Orientale, Alessandria, Italy.* [b]*IBR, Austrian Academy of Science, Graz, Austria.* E-mail: gianluca.croce@mfn.unipmn.it

Marine sponges deposit hydrated silica in needle-like objects called spicules. These spicules also contain a protein axial filament which functions as template for silica deposition.

This presentation deals with the fiber diffraction structural study of the organization of the axial filaments in spicules from different sponges, carried out using a SAXS setup with synchrotron radiation. The collected images show diffraction spots sharper than what can be expected from a regular polymeric fiber, indicating that the protein units in the spicule axial filaments must form highly ordered patterns. The analysis of the position and distribution of the spots reveals a hexagonal arrangement with different possible bi- and tri-dimensional dispositions of the units along the main axis of the spicules. Analysis after thermal treatments reveals a structural ordering accompanying the thermal degradation of the organic material. This confirms our hypothesis that the protein units act as template in the formation of an inorganic mesoporous structure.

Our results suggest the following possible mechanism for the biosilification process in spicules. The initial step consists in the formation of a very ordered disposition of the protein units, forming a regular mesoporous arrangement in a silica matrix, similar to that found in synthetic materials. In a second step the biosilification process continues with a deposition of amorphous silica on the outer walls of the mesoporous core.

Keywords: biomineralization, SAXS, porous materials

MS98 ANALYSIS OF ANISOTROPIC MATERIALS

Chairpersons: Yoshiyuki Amemiya, Iris Torriani

MS98.30.1

Acta Cryst. (2005). A**61**, C123

Polymer and Biopolymer Microstructure Analysis by Scanning SAXS/WAXS

Christian Riekel, *European Synchrotron Radiation Facility, B.P.220, F-38043 Grenoble Cedex, France.* E-mail: riekel@esrf.fr

Scanning SAXS/WAXS using a 2D-detector provides an "images" of a bulk structure with each "pixel" of the image containing information from the unit-cell to morphological scales. Source and instrumental developments at 3rd generation synchrotron radiation sources allow routine use of micron- and submicron-sized X-ray beams extending currently to about 100 nm. The choice of beam size requires, however, usually a compromise on the low-angle resolution. For X-ray microbeams, polymers and biopolymer fibres often show lateral heterogeneities such as skin-core structures. Although such heterogeneities have already been known from electron microscopy/diffraction studies, the interest in scanning SAXS/WAXS is the possibility of performing in-situ studies during deformation of bulk samples. A number of examples from high performance polymer fibres will be reviewed. In-situ experiments are usually performed at room temperature, which poses particular problems due to radiation damage as will be shown for the hydration of starch granules.

Keywords: X-ray microdiffraction, synchrotron radiation, polymers

MS98.30.2

Acta Cryst. (2005). A**61**, C123-C124

Small-angle X-ray Scattering Analysis of Anisotropic Block Copolymer Microdomains

Takeji Hashimoto, *Department of Polymer Chemistry, Kyoto University, Katsura, Kyoto 615-8510, and Advanced Science Research Center, JAERI, Tokai-mura, Ibaraki Pref. 319-1195, Japan.* E-mail: hashimoto@alloy.polym.kyoto-u.ac.jp

We present small-angle x-ray scattering (SAXS) analysis of anisotropic block copolymer materials of polystyrene-*block*-polyisoprene-*block*-polystyrene (SIS) as a fundamental statistical mechanical problem for open nonequilibrium systems. In order to develop the anisotropy we applied a large amplitude oscillatory shear strain to the system. We analyzed, at *real-time* and *in-situ,* time-evolution of the anisotropic spatial arrangement of microdomains by using time (strain-phase) resolved SAXS. We shall first discuss the