also a potent inhibitor of (Zn/Zn)bLAP. This combined approach provided insights on interaction of bLAP with sulphydryl-containing compounds, showing that metal exchange in site 1 modulates binding to these molecules that, depending on metal nature, may result as enzyme substrates or inhibitors.

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Keywords: biocrystallography of protein, proteins-inhibitor complexes, metalloenzymes

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The Crystal Structure of Human CA II Bound to a Strong Benzenesulfonamide Inhibitor

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Carbonic anhydrases (CAs) are ubiquitous metalloenzymes present in prokaryotes and eukaryotes, which catalyze the reversible hydration of CO_2. In previous studies we have investigated by means of X-ray crystallography the rational design of sulfonamide/sulfamate/sulfamide inhibitors of this enzyme, which is involved in a multitude of physiological and pathological processes [1]. At least fourteen different CA isozymes are presently known in humans, and many of them are targets for the design of inhibitors with potential use as antiglaucoma, anti-obesity, or anticancer drugs among others. A class of CA inhibitors which showed very promising applications are the thioureas obtained from isothiocyanato sulfonamides and amines, hydrazines or amino acids. Such compounds generally showed potent inhibitory activity against the human cytosolic isozyme CA II as well as the transmembrane, tumor-associated isozyme CA IX, being thus interesting candidates for developing antiglaucoma/antitumor therapies based on them.

Here we report the first X-ray crystal structure of a thioureido-benzensulfonamide derivative in complex with human CA II as well as its inhibitory properties against isozymes I, II and IX [2].

[1] Supuran C.T., Scozzafava A., Casini A., *Med. Res. Rev*., 2003, **23**, 146. [2] Di Fiore A., De Simone G., Menchise V., Pedone C., Casini A., Scozzafava A., Supuran C.T., *Bioorg. Med. Chem. Lett*., 2005, *in press.*

Keywords: biocrystallography of protein, protein-inhibitor complexes, rational inhibitor design

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Role of the Non-protein Ligand at the Ni-Fe Active Site of [NiFe] Hydrogenase

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Hydrogenases catalyze oxidoreduction of molecular hydrogen and have a potential value for a use of dihydrogen as an energy source.

[NiFe] hydrogenase possesses two oxidized states, Ni-A (inactive) and Ni-B (active). The pure Ni-A state was successfully prepared from the solution of the as-purified enzyme (mixture of the Ni-A and Ni-B states), and the crystal structures of both the Ni-A and Ni-B states have been determined at ultra-high resolution. The shape and size of the electron densities show that Ni-B possesses a monatomic non-protein bridging ligand between the Ni and Fe atoms at the active site and the cysteine sulfur ligand (Cys546) was modified by unknown atomic species (X546). Whereas Ni-A has a diatomic ligand at the bridging site and two systein sulfur ligands (Cys546 and Cys84) were also modified by unknown species (X546 and X84). X546 of Ni-A was shifted towards the Ni atom about 1.0 Å compaired to that of Ni-B. Diatomic bridging ligand and X84 of Ni-A seem to block the pathway of dihydrogen.

The essential features of the enzyme structure at the resting state and the transition mechanism from Ni-B to Ni-A are proposed.

Keywords: [NiFe] hydrogenase, Ni-A and Ni-B, non-protein ligand

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Crystal Structure of Mouse Carnosinase CN2 at 1.8 Å Resolution

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L-Carnosine, β-alanyl L-histidine, is found as a bioactive dipeptide which affects autonomic neurotransmission and blood pressure through histamenergic nerves and is present in mammalian tissues including the central nervous system. In mammals, two types of carnosinases, CN1 and CN2, both of which catalyse the hydrolysis of L-carnosine, with different properties are known. The mouse carnosinase CN2 was found to be highly concentrated in the parafascicular nucleus of the thalamus and so on in the brain, which suggests carnosine is degraded by CN2 to supply the substrate of histamine-synthesizing enzyme, histidine decarboxylase. We started crystallographic study of CN2 from mice to understand its enzyme mechanisms on a structural basis.

The MAD data were collected on beamline BL6A of the Photon Factory using an ADSC Quantum 4D CCD detector. The protein phases were determined with the program Sharp and improved with the program dm using non-crystallographic symmetry. The peptide model was built with the program ARP/wARP. The structure is now being refined with the program Refmac5.

[1] Otani H., Okumura N., Hashida-Okumura A., Nagai K., *J. Biochem.*, 2005, **137**, 167.

Keywords: metalloproteinases, enzyme active site, protein structure determination

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Class III Superoxide Reductase from *Treponema pallidum*

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Superoxide reductase of *Treponema pallidum* (*Tp* SOR) is a metalloprotein responsible for the scavenging of superoxide radicals in the cell [1]. SORs can be divided into three classes according to amino acid sequence alignment: Members of class I have only the catalytic domain. Class II and III SORs present an additional N-terminal domain that, in the case of class II, has an additional non-heme iron center ($Fe(Cys)_4$) of the rubredoxin type [2]. The active site, common to all three classes, is an iron center, ($Fe(Cys)(His)_4$) that reacts with superoxide in the reduced state.

*Tp*SOR is the first member of class III to be structurally characterized. Blue crystals of the oxidized form diffracted beyond 1.55 Å. A highly redundant in-house data set allowed solving the structure and synchrotron data led to phase improvement.

[1] Jovanović T., Ascenso C., Hazlett K.R., Sikkink R., Krebs C., Litwiller R., Benson L.M., Moura I., Moura J.J.G., Radolf J.D., Huynh B.H., Naylor S., Rusnak F., *J. Biol. Chem.*, 2000, **275**, 28439-28448. [2] Archer M., Huber R., Tavares P., Moura I., Moura J.J.G., Carrondo M.A., Sieker L.C., LeGall J., Romão M.J., *J. Mol. Biol*., 1995, **251**, 690-702.

Keywords: superoxide, soft X-rays, iron

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Crystal Structures of Cytochrome c Peroxidases from *Ps. nautica* and *Ps. stutzeri*

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