

PS04.02.19 CRYSTAL STRUCTURE OF THE WIDE-SPECTRUM BINUCLEAR ZINC β -LACTAMASE FROM BACTEROIDES FRAGILIS. Nestor O. Concha¹, Beth A. Rasmussen², Karen Bush², and Osnat Herzberg¹. ¹Center for Advanced Research in Biotechnology, University of Maryland, Rockville, MD. ²Wyeth-Ayerst Research, Lederle Laboratories, Pearl River, NY

The metallo β -lactamase from *Bacteroides fragilis* hydrolyses a wide range of β -lactam antibiotics, and is not susceptible to any known β -lactamase inhibitor. The proliferation of the *B. fragilis* bacteria in nosocomial infections, and the report of plasmid-mediated dissemination of the enzyme, underscore the urgency of acquiring structural information for the development of new therapeutic agents. The crystal structure of the enzyme was determined using multiwavelength anomalous diffraction at the zinc absorption edge, and subsequently refined at 1.85 Å resolution. The enzyme folds into a four layer $\alpha/\beta/\beta/\alpha$ structure with the active site located at the edge of the β -sandwich. The molecule contains a binuclear (Zn²⁺-Zn²⁺) center with one zinc in a tetrahedral coordination, and the other in a pentagonal coordination. A water molecule is shared by both zinc ions, indicating that it is a hydroxide. A second water binds only to the pentagonally coordinated zinc. A variety of bound substrates can be modeled in the active site, providing the structural basis for the wide-spectrum profile of the *B. fragilis* β -lactamase. The active site architecture and the models of the bound substrates lead to the proposed catalytic mechanism in which the hydroxide is assumed to be the nucleophilic group. An alternative proposal in which the water molecule that binds to the pentagonally coordinated zinc plays the nucleophilic role is less favored, but cannot be ruled out.

PS04.02.20 ANALYSIS OF THE VANADIUM DEPENDENT HALOPEROXIDASE FROM CORALLINA OFFICINALIS Andrew Dalby¹, Cliff Rush¹, Andrew Willetts¹, Gideon Davies², Zbigniew Dauter³, Jennifer Littlechild¹. ¹Departments of Chemistry and Biological Science, University of Exeter UK., ²Department of Chemistry, University of York, UK., ³EMBL, DESY, Germany.

Crystals have been grown of the vanadium dependent haloperoxidase from *Corallina officinalis*. The protein is a dodecamer with a subunit mass of 64 kdaltons. Electron microscopy of the similar enzyme from *Corallina pilulifera* has suggested that it is composed of two stacked hexamers. The enzyme contains no haem and is dependent on vanadium for activity and is particularly thermostable and resistant to organic solvents. It therefore has useful applications in bio-transformations.

Crystals were grown from polyethylene glycol (PEG) 6,000 and 0.4 M potassium chloride by vapour diffusion. Data was collected at the EMBL Hamburg outstation on beamline X11 at a wavelength of 0.92 Å. The crystals diffract beyond 2.0 Å. A data set has been collected to 3.15 Å that is 98 % complete and with an R-merge of 6.5%.

The cell parameters are cubic with $a = b = c = 310$ Å, and the space group is either I23 or I213. This would infer that there are eight molecules per asymmetric unit with a Matthews coefficient of 2.3 Å³/dalton.

With so many molecules in the asymmetric unit the determination of this structure will involve the use of ncs averaging. Currently further native data to higher resolution is being collected.

PS04.02.21 CRYSTALLIZATION AND PRELIMINARY X-RAY ANALYSIS OF A NITRATE REDUCTASE FROM DESULFOVIBRIO DESULFURICANS ATCC 27774. J. M. Dias¹, C. Carneiro², M. J. Almendra², I. Moura², J. Moura², J. LeGall^{1,3}, M. J. Romco^{1,4}. ¹ITQB, Apt 127, 2780 Oeiras, Portugal; ²FCT-UNL, 2785 Monte Caparica, Portugal; ³University of Georgia, Athens, GA 30602, USA; ⁴IST, Dept. Qummica, 1096 Lisboa Codex, Portugal

A nitrate reductase from *Desulfovibrio (D.) desulfuricans* ATCC 27774 (a sulfate reducing bacteria, that can adapt to nitrate respiration) has been isolated, allowing the elucidation of the enzymatic system required to convert nitrate (through nitrite) to ammonia. *D. desulfuricans* ATCC 27774 nitrate reductase is a monomeric periplasmic (soluble) enzyme with 74 kDa, containing one [4Fe-4S] centre and one molybdenum atom per molecule [1].

Crystals of this nitrate reductase were obtained by vapour diffusion with hanging drop technique using 6% (m/v) PEG 10 K as precipitant at pH 6.5 with 0.1M MES. The crystals grow at 20°C or 4°C to an approximate size of 0.3 x 0.3 x 0.3 mm.

The crystals diffract beyond 3.15 Å resolution. Native data sets were collected using a MAR-Research Imaging Plate area detector and graphite monochromated Cu-K α radiation from an Enraf Nonius rotating anode generator operated at 45kV and 99 mA. Data were processed with Denzo and the space group is P3₁21 with cell parameters $a=b=106.9$ Å, $c=138.1$ Å.

The data set comprised 13201 measurements of 9695 independent reflections in the resolution range $30.0 < d < 3.15$ Å, with an overall completeness of 56.9 %, Rmerge(I)= 11.8 %.

1 - Bursakov, S., Liu, M., Payne, W., LeGall, J., Moura, I., Moura, J., Anaerobe, 1, 55-60, (1995).

PS04.02.22 CRYSTAL STRUCTURE OF A METALLO β -LACTAMASE II FROM *B. CEREUS* AT 2.5Å. Stella Maris Fabiane[§], M. Sohi[§], T. Wan[§], D. J. Payne[¶], J. H. Bateson[‡], T. Mitchell[‡], B. J. Sutton[§], [§]King's College London, 26-29 Drury Lane, London WC2B 5RL, UK, [¶]SmithKline Beecham Pharmaceuticals, 1250 South Collegeville Rd, P. O. Box 5089, Collegeville, PA 19426-0989, USA, [‡]SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ, UK

Crystals of the Zn-dependent β -lactamase ('cephalosporinase') from *Bacillus cereus* 569/H were grown using the vapour diffusion technique. They belong to space group P3₁21, with cell dimensions $a=b=68.14$ Å, $c=180.68$ Å, with two enzyme molecules per asymmetric unit. The structure of the enzyme was solved by multiple isomorphous replacement and solvent flattening techniques and refined to 2.5 Å. The final crystallographic R factor is 19.2% ($R_{\text{free}} = 26.8\%$).

The structure reveals an arrangement of secondary structural elements very similar to the N-terminal nucleophile amidohydrolases. The intermolecular interactions between the non-crystallographically related molecules involve head-to-tail packing of α -helices, and a continuous β -sheet extending across the interface. The active site is flanked by a disordered loop that may be important for the mechanism; cryocrystallographic studies are in progress to investigate its role in substrate binding.