PS04.01.80 STRUCTURAL INVESTIGATIONS ON COFACTOR-FREE HALOPEROXIDASES. H. J. Hecht[1], B. Hofmann[1], J. Altenbucher[2], K. H. van Pée[3], [1]GBF (Gesellschaft für Biotechnologische Forschung),Dept. MSF, Mascheroder Weg 1, D-38124 Braunschweig, FRG, [2]Univ. Stuttgart, Inst. Indust. Genetik, D-70569 Stuttgart, FRG, [3]TU Dresden, Inst. Biochemie, D-01062 Dresden, FRG

Haloperoxidases catalyse the halogenation of organic compounds in the presence of halide ions and peroxides like H_2O_2. The bacterial haloperoxidases catalyse this redox reaction without the involvement of a known cofactor[1,2]. In order to elucidate the reaction mechanism recently the crystal structure of the cofactor-independent haloperoxidase CPO-A2 from the 7-chloro-tetracycline- producing *Streptomyces aureofaciens* ATCC 10762 was solved by MIR[3]. The overall structure of this haloperoxidase has been characterized as a α/ß-hydrolase fold with a catalytic triad in the active center consisting of Ser 98, His 257 and Asp 228, but the exact mechanism of the enzyme remained still unclear. The halogenation reaction of these haloperoxidases requires the presence of organic acids like acetate or propionate. Therefore as reaction mechanism it was postulated, that acetate, activated by the nucleophile Ser O_γ, could be oxidised by peroxide to the peroxoacid in a first step, which then could oxidize the halide ion, probably with the involvement of as yet unidentified additional residues in the active center.

Crystals of haloperoxidase CPO-A2 were obtained at pH 8.0, where the enzyme shows only low residual activity[3]. As part of this investigation of the reaction mechanism we determined the structures of the related haloperoxidase from *Streptomyces lividans* (CPO-L) both at pH 8.0 and at pH 6.0, the activity pH optimum. The role of M99 in CPO-A2 was investigated with the CPO-A2 mutant Met99—>Thr which is analogous in this position to CPO-L but inactive. The structure of this mutant has also been determined and refined at 1.5 Å. We present here these three structures and discuss their differences and the implications for the reaction mechanism.

CPO-A2 M99T from *Streptomyces aureofaciens* crystallizes from 1.8 M ammonium sulphate at pH 8.0. Crystals diffract to 1.5 Å resolution, belong to spacegroup I23 with a = 121.7 Å and contain one monomer in the asymmetric unit. CPO- L from *Streptomyces lividans* TK64 crystallizes at pH 8.0 from 2.1 M ammonium sulphate in the space group I4 with a = 176.5 Å, c = 64.0 Å. Crystals diffract to 1.9 Å and contain one trimer per asymmetric unit. At pH 6.0 CPO-L crystals grow from 2.0 M ammonium sulphate in space group $P2_12_12_1$ and diffract to 2.6 Å.The structures have been solved by molecular replacement using AMORE and have been refined using X-PLOR.

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PS04.01.81 HORSERADISH PEROXIDASE C*: CRYSTAL STRUCTURE WITH AND WITHOUT THE SUBSTRATE: BENZENE HYDROXAMINE ACID (BHA) BOUND IN THE ACTIVE SITE. M. Gajhede, A. Henriksen, Centre for Crystallographic Studies, Department of Chemistry, The H.C. Orsted Institute, University of Copenhagen, DK-2100 Copenhagen, Denmark, D.J. Schuller, Department of Molecular Biology & Biochemistry, University of California, Irvine California USA & A.T. Smith, Biochemistry Laboratory, School of Biological Sciences, University of Sussex, Brighton BN1 9QG, England

When focusing on Heme-containing proteins, the three-dimensional structure is known for quite a few: A large number of members of the Cytochrome c and Globin-like superfamilies, members of the superfamily of Heme-linked catalases and members from the superfamily of Heme-dependent peroxidases. However there is still one very important class of structures of heme-containing proteins that has been unknown until just recently. This is the class of plant peroxidases. Now however the structures of peanut peroxidase (Schuller D.J., Ban N., van Huystee, R.B., McPherson A. & Poulos T.L. (1996) Structure, in press), barley peroxidase (Henriksen A., Welinder K.G. & Gajhede M. in preparation) and recombinant horseradish peroxidase C (HRPC*) (Gajhede M., Schuller D.J, Henriksen A., Smith A.T. & Poulos T.L. in preparation) have been solved. The structure of HRPC* has been solved by molecular replacement, using the peanut peroxidase structure as a model. The structure has been solved in two crystal forms: with and without the substrate benzene hydroxamine acid bound in the active site. A detailed analysis of the two structures will be presented.

PS04.01.82 ADAPTATION TO EXTREME SALT ENVIRONMENT: THE STRUCTURE OF DIHYDROFOLATE REDUCTASE FROM HALOFERAX VOLCANII. Ursula Pieper, Geeta Kapadia, Moshe Mevarech and Osnat Herzberg, Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, 9600 Gudelsky Drive, Rockville MD 20850, USA

Haloferax volcanii is a halophilic archaeon, native in the dead sea, that adapted to high salt concentrations by accumulating up to 4M KCl inside the cytoplasmic membrane. Amino acid sequence analysis of a number of halophilic proteins reveals an enrichment of negatively charged amino acids which have been proposed to solvate the protein more efficiently.

We present here the crystal structure of dihydrofolate reductase from *Haloferax volcanii* (*h*-DHFR), the first structure of a protein originated from this organism and the third structure of a halophilic protein. The structure has been determined by molecular replacement using as a search model the superpositions of seven DHFR coordinate sets available in the PDB. Subsequently, the structure has been refined at 2.6 Å resolution to an R-value of 0.18.

The overall structure of *h*-DHFR is very similar to the other known DHFR structures. The largest differences occur in one helix and a loop region involved in NADP binding. There are no obvious features that correlate with the enzyme functioning at high salt. This is in contrast to the report of unusual charge clusters found in malate dehydrogenase from *Haloarcula marismortui*. For providing structural basis to the functional behavior, we argue that it is not the ability to function in high salt that needs explaining, because many other mesophilic proteins function well at high salt. Rather, the impaired function at low salt should be rationalized. In that respect, *h*-DHFR may not be a genuine extreme halophilic enzyme, because it is active at rather low salt concentrations. Nevertheless a number of subtle differences in both the amino acid composition and tertiary structure are observed and together they may account for the halophilic behavior.