mutants of active site residues of AnAEst were determined. While the wild-type enzyme showed highest catalytic efficiency for naphthyl esters relative to phenyl esters, the R54G mutant displayed a 2.4 fold increase in catalytic efficiency for phenyl esters over naphthyl esters. The kinetic studies in conjunction with docking studies confirm the structural role of Arg54 and other active site residues in both substrate binding and catalysis.

[1] C.C. Akoh, G.C. Lee, Y.C. Liaw, T.H. Huang, J.F. Shaw, *Prog. Lipid Res*. **2004**, *43*, 534–552. [2] K. Bakshy, S.N. Gummadi, N. Manoj, *Biochim Biophys Acta* **2009**, *1794*, 324-334.

Keywords: enzyme, kinetics, esterase

MS93.P12

Acta Cryst. (2011) A**67,** C772

Structural insight into iron pathways in ferritin

Stefano Mangani,[a,b] Ivano Bertini,[b] Daniela Lalli,[b] Cecilia Pozzi,[a] Camilla Rosa,[b] Paola Turano,[b] [a]*Department of Chemistry, University of Siena, Siena (Italy).* [b]*Magnetic Resonance Center (CERM) and Department of Chemistry-University of Florence (Italy).* E-mail: mangani@unisi.it

Ferritin directs the reversible biomineralization of iron. Crystals of apoferritin loaded, in aerobic conditions, with different amounts of $FeSO_4$ and $CuSO_4$ were studied by X-ray crystallography and the structure of the tripositive iron and bipositive copper adducts were determined at 2.7 Å and 2.8 Å resolution, respectively.

Anomalous diffraction experiments reveal the binding of the iron substrate to the ferroxidase site and to other sites in the protein, including a possible nucleation site for the iron mineral.

The metal coordination sphere at the catalytic site is redox dependent. The differences between iron and copper binding provide clues on the reaction mechanism and on the path of iron from the so-called C3 pore (entrance) to the C4 pore (exit into the cavity) through the catalytic site.

The crystallographic data combined with the previously reported results from NMR experiments, magnetic susceptibility measurements and other crystallographic determinations on different metal adducts [1], [2], [3], provide an updated model for the iron processing by ferritin.

[1] P. Turano, D. Lalli, I.C. Felli, E.C. Theil, I. Bertini, *Proc.Natl.Acad.Sci. USA* **2010**, *107*, 545–550. [2] M. Matzapetakis, P. Turano, E.C. Theil, I. Bertini, *J.Biomol. NMR* **2007**, *38*, 237–242. [3] T. Tosha, H.L. Ng, O. Bhattasali, T. Alber, E.C. Theil *J Am Chem Soc*. **2010,** *132*, 14562-14569.

Keywords: biomineralization, iron, anomalous diffraction

MS93.P13

Acta Cryst. (2011) A**67,** C772

Structural comparison of the milk-clotting enzymes bovine and camel chymosin

Jesper Langholm Jensen,[a,b] Anne Mølgaard,[a] Jens-Christian Navarro Poulsen,[a] Hans van den Brink,[b] Marianne Harboe,[b] Jens Bæk Simonsen,[c] Karsten Bruun Qvist,[d] and Sine Larsen,[a] [a]*Department of Chemistry, University of Copenhagen, Copenhagen, (Denmark).* [b]*Department of Enzymes, Chr. Hansen A/S, Hørsholm, (Denmark).*[c]*Department of Basic Sciences and Environment/Biophysics, University of Copenhagen, Copenhagen, (Denmark).* [d]*Innovation, Chr. Hansen A/S, Hørsholm, (Denmark).* E-mail: langholm@chem.ku.dk

Chymosin is an aspartic protease that clots milk, thus initiating cheese formation. Cows are the major source for milk, but contrary to what would be expected, bovine chymosin is not the best milk-clotting enzyme known – camel chymosin is better[1]! This surprising observation initiated this project to determine the cause of this behaviour.

The camel chymosin obtained from expression in *Aspergillus niger* has been examined. Six variants have been separated using hydrophobic interaction chromatography. The variants differ with respect to glycosylation, activity, and other, at this point, unknown properties.

The structure of the double glycosylated camel chymosin has been solved. A good comparison with bovine chymosin requires the availability of a good experimental data set. However, models but no experimental data have been deposited for the previously available structures. Therefore the structure of bovine chymosin has been determined to a higher resolution (1.8 Å) than those previously available.

The overall fold of bovine and camel chymosin is similar, however camel chymosin is found in a self-inhibited state, in which the N-terminal blocks the binding cleft. The conformation commonly seen in other aspartic proteases appears to be destabilized by charge and sterical differences of the N-terminal. The resuspended crystals of camel chymosin show milk-clotting activity, hence other conformations are available, in which the N-terminal is located outside the binding cleft. Chymosin's interactions with its inhibitors and substrates are being investigated. The structure of bovine chymosin in complex with the aspartic protease inhibitor pepstatin has been solved. The structural results will be discussed in relation to the differences in the milk-clotting properties of bovine and camel chymosin.

[1] S.R. Kappeler, H.M. van den Brink, H. Rahbek-Nielsen, Z. Faraha, Z. Puhana, E.B. Hansen, E. Johansen. *Biochem. Biophys. Res. Comm* **2006**, *342(2)*, 647-654.

Keywords: protease activity selectivity

MS93.P14

Acta Cryst. (2011) A**67,** C772-C773

Unveiling the substrate-bound structure of a Baeyer-Villiger Monooxygenase

Brahm J. Yachnin,[a] Peter C. K. Lau,[b] Albert M. Berghuis,[a] [a]*Department of Biochemistry, McGill University, Montreal, (Canada).* [b]*Biotechnology Research Institute, National Research Council of Canada, Montreal, (Canada).* E-mail: brahm.yachnin@mcgill.ca

The high specificity, efficiency, and "green" properties of biocatalysts make them increasingly attractive alternatives to conventional chemical catalysts. The Baeyer-Villiger monooxygenases (BVMOs), which catalyze the synthetically useful Baeyer-Villiger oxidation reaction, are a promising class of biocatalysts. The broad substrate spectrum of these flavoproteins makes them particularly suited for use in industry, allowing them to be engineered for specific applications. These enzymes have an FAD cofactor, and use molecular oxygen and NADPH to convert a ketone to an ester. In spite of a great deal of research characterizing these enzymes, most notably cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp. NCIMB 9871, very little structural information is available. Thus far, one crystal structure of phenylacetone monooxygenase in complex with the FAD cofactor [1] and two crystal structures of CHMO from *Rhodococcus* sp. HI-31 in complex with both FAD and $NADP^+$ [2] have been published. These three structures revealed some of the overall domain movements that are required for BVMOs to function. In particular, a large rotation in the NADPH-binding domain was revealed to cause the sliding in of the