	Experiment title: Carboxylesterase (EST2) from the thermophilic *A. acidocaldarius*	**Experiment number**:
Beamline: ID14-1	**Date of experiment**: from: 11-02-2002 to: 12-02-2002	**Date of report**: 26/7/02
Shifts: 1	**Local contact(s)**: Sigrid KOZIELSKI	*Received at ESRF:*

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Report:

Esterases, lipases and cholinesterases belong to a large family of phylogenetically related proteins with representatives in the domains of *Eukarya*, *Bacteria* and *Archaea*. On the basis of their amino acid sequence homology and the occurrence of different conserved motifs, they are generally classified in three separate groups: C, L and H. In the past, structural investigations focused on proteins belonging to the C and L groups, revealing the common topological α/β-hydrolase fold, well known among lipases.

The first structural information on the H subfamily were obtained only in the 1999 from the crystal structure resolution of Brefeldin A esterase (BFAE) from *B. subtilis* [1]. More recently, De Simone and coworkers [2] reported the x-ray crystal structure of a new thermophilic carboxylesterase from *A. acidocaldarius* (EST2). The analysis of this structure allowed a detailed characterization of the EST2 active site with the identification of the residues involved in the formation of the oxyanion hole and of the hydrophobic acyl binding pocket. Apart from this, the structural comparison between EST2 and BFAE allowed to identify the possible determinants for thermal stability within this class of enzymes.

In order to get more insights in the understanding of the structure-function relationships for these proteins, an EST2 mutant (Mut18S) has been designed and expressed. In particular, the residue Gly84, which was involved in the formation of the oxyanion hole, was substituted with a Serine.

The G84S mutation led to a drammatic change in the EST2 specificity, as well as in its thermostability. Mut18S has been, thus, crystallized and a complete dataset to 2.0 Å resolution, has been collected at the ESRFon the beamline ID14-1. Diffracted intensities were processed, using the HKL crystallographic data reduction package

(Denzo/Scalepack). Data processing statistics are given in Table 1. The structures was solved with molecular replacement; the X-ray structure of EST2 (pdb code 1evq) has been used as starting model. The refinement and the analysis of the structure is still in progress.

Table 1. Crystal and data collection parameters.

Crystal	**MUTG**
Space group	P21212
a (Å)	63.66
b (Å)	82.17
c (Å)	50.22
Independent molecules	1
Resolution limits (Å)	20-2.0
Temperature (K)	100
Total reflection	319571
Independent reflection	17662
Completeness (%)	
Overall	97.8
Last resolution shell	80.4
R-merge*	
Overall	0.056
Last resolution shel	0.137
I/sigma(I)	
Overall	17.7
Last resolution shell	6.8

*$R_{merge} = \Sigma|I_i - \langle I \rangle| / \Sigma I_i$; over all reflections

REFERENCES

1. Wei, Y., Contreras, J.A., Sheffield, P., Østerlund, T., Derewenda, U., Kneusel, R.E., Matern, U., Holm, C. & Derewenda, Z.S. (1999). *Nat. Struc. Biol.* **6,** 340-345.
2. De Simone, G, Galdiero, S, Manco, G, Lang, D, Rossi, M., and Pedone, C. (2000) *J. Mol. Biol.*,**303**, 761-771.
3 G. De Simone, V. Menchise, G. Manco, L. Mandrich, N. Sorrentino, D. Lang, M. Rossi and C. Pedone. (2001) *J. Mol Biol.*, 714, 507-518.