ESRF	**Experiment title:** Structure determination of leukotriene A4 hydrolase	**Experiment number:** LS 1215
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1 Report:

Leukotrienes are lipid mediators involved in inflammatory and allergic processes. Leukotriene B_4 is regarded as the most important mediator of inflammation (Samuelsson *et al.,* 1987) and has been detected in large amounts in diseases such as arthritis and gout. The compound is formed from arachidonic acid via the sequential action of two enzymes. 5-lipoxygenase catalyzes the formation of the epoxide intermediate LTA4 which is in turn converted into LTB4 by the enzyme LTA4 hydrolase. In addition to its epoxide hydrolase activity, LTA4 hydrolase also has peptidase/amidase activity towards synthetic substrates. Although site directed mutagenesis work has identified residues involved in the amino-peptidase activity, much less is known about the epxide hydrolase activity. It has been shown that that the active sites for the peptidase and epoxide hydrolase activities are overlapping but. not identical.

To reveal the molecular mechanism of this bifunctional protein, its interactions with the substrate and for the design of potential anti-inflammation and anti-allergic drugs we determined the structure of this enzyme.

Crystals were grown using liquid-liquid difussion in capillaries. For this 5 ml 15% PEG8000, 100 mM Na-actetate, 100 mM imidazole buffer pH 6.8, 2.5 mM $YbCl_3$ is injected into the bottom of a melting point capillary. On top of this 5 ml 5mg/ml LTA, hydrolase in 10 mM Tris buffer pH 8.0 + 1mM bestatin is layered and the capillary is closed and stored at room temperature. The crystals grow in 3 to 4 weeks and belong to space-group $P2_12_12$ with cell dimensions a = 67.59 A, b = 133.51 A, c = 83.40 Å, $\alpha = \beta = \gamma = 90°$ at 100K.

The structure was determined by using multiple anomalous dispersion measurements on the L_{III} edge of Ytterbium (1 = 1.3862A) at beamline BM14 in the ESRF, Grenoble. 3 datasets , peak (PK), point of inflection (PI) and hard remote (RM) were collected to 2.5Å resolution from the same crystal. The crystal was aligned such that Bijvoet equivalent could be collected in one pass of 90° for each wave-length. For the RM a subsequent dataset to, 2.15Å was collected. A second crystal was used for obtaining a dataset to 1.95A. (For statistics on data-collection and quality, see table 1). Data were integrated using the program Denzo scaled to each other using Scalepack and further data analysis was performed using programs from the CCP4 package.

From Patterson functions one ma.jor and one minor Yb positions could readily be identified, a third position was identified during heavy atom refinement in difference Fourier maps. The heavy atom parameters were refined using MLPHARE and SHARP (for details see table 1). The final FOM was 0.57 to 2.15Å. Phase information was further improved to 2.15Å by using SOLOMON with a solvent contend of 43%. The maps had very good quality and the full protein molecule (res. 1 to 610) could be traced unambiguously. The protein is folded into three domains; an N-terminal, a central catalytic and a C-terminal domain which together form a flat triangular arrangement. Between the three domains a deep cleft is formed which presuably accomodates the lipid substrate and at the bottom of the cleft the Zn2+ binding site is located. The catalytic domain shares a surprising structural homolgy with thermolysin although the sequence identity along this segment is minimal. We are currently refining this model.

Table 1. Datacollection and heavy atom refinement.

	Crystal 2		Crystal 1	
diffraction limit (Å)	1.95	2.5	2.5	2.15
nature of wavelength		f" peak	point of inflection	hard remote
l (Å)	0.992	1.3838	1.3842	0.8856
Rmerge(I) (%)	4.3	3.0	3.0	3.0
Ranom (I) (%)	4.4	8.1	4.7	3.3
Completeness (%)	98.2	97.7	97.7	97.8
Mean I/s(I)	13.3	6.59	10.74	13.16
Multiplicity of observation	4.7	3.3	6.2	3.8
Riso (F) (%)		2.4	-	7.0
Phasing power isomorphous		-/-	3.7/5.4	3.5/5.4
Phasing power anomalous		-13.2	-/4.7	-/3.8
Rcullis isomorphous		-/-	0.37/0.38	0.33/0.36
Rcullis anomalous		– / 0.60	- / 0.40	- / 0.53