ESRF	**Experiment title:** Acetohydroxyacid isomeroreductase : high resolution measurements in view of time resolved studies	**Experiment number:** LS 894
Beamline: BM14	**Date of experiment:** from: 28-02 to: 01-03-1998	**Date of report:** 13-05-98
Shifts: 6	**Local contact(s):** Vivian Stojanoff	*Received at ESRF:* 2 4 JUIN 1998

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Report:

Acetohydroxyacid isomeroreductase catalyses a two step reaction: an isomerisation followed by a reduction. Both steps are highly dependent upon divalent cations, and the second step requires NADPH. The enzyme has a strong preference for magnesium compared to other cations, and displays residual activity (3%) with manganese (Dumas et al., 1995). We have high resolution refined structures of the following complexes :

i) isomeroreductase - Mg++ - NADPH - inhibitor IpOHA (1.65Å resolution, Biou et al., 1997) ii) isomeroreductase - Mn++ - NADPH - substrate AHB (1.60Å resolution, Thomazeau et al., in preparation). This last complex shows the reaction product in the active site.

In vitro tests performed on the enzyme with **nickel** and **zinc** showed no measurable activity. Therefore we cocrystallized the enzyme with those cations and the substrate.

Measurement conditions We were allocated six shifts on BM14. Those shifts were planned during a 16 bunch run, but for technical reasons, the machine was run in 2/3 filling mode during those 2 days, which gave us more intensity. The detector was a MarResearch 345 imaging plate.

Data collections We collected data on the Ni^{++} and Zn^{++} complexes, then soaked one Zn++ crystal into a Mg^{++} solution and performed measurements at the Zn edge, in order to be able to identify the cation in the active site.

Experiments Performed :

Cation	Ni++	Zn++	Zn replaced by Mg
Wavelength	0.83Å	0.83Å	1.24Å (Zn edge)
Unit cell	112.5, 112.5, 338.6, 90, 90, 120.	65.6, 90.6, 183.7, 90, 90, 90.	65.7, 90.7, 183.9, 90, 90, 90.
Space group	$P3_121$	$P2_12_12$	$P2_12_12$
Resolution	2.581 (detector edge)	2.0481 (detector edge)	2.581 (detector edge)
R-merge (at res. edge)	0.049 (0.173)	0.047 (0.098)	0.043 (0.084)
Molecular replacement R-factor	0.326 (30-4Å)	0.46 (30-2Å)	0.36 (30-3Å)
Comments	reaction product in active site	reaction product in active site	reaction product in active site

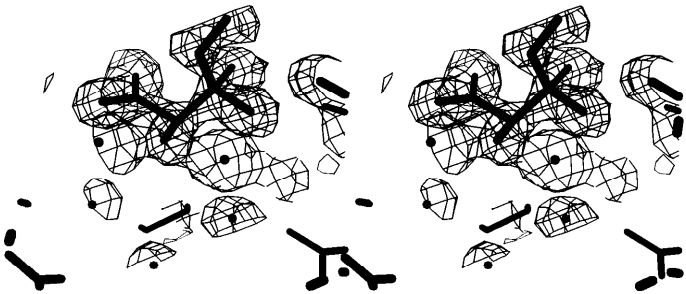

Figure 1 Stereo view of acetohydroxyacid isomeroreductase active site. The map is a 2fo-fc, calculated using the Ni amplitudes and model phases from a previous structure (K. Thomazeau et *al.*, in preparation), contoured at 1 sigma level. It is obvious that the ethyl substituant is on the rightmost carbon atom and not on the central one, showing that the isomerisation has occurred.

In summary, we collected good quality data on three different crystals. However, our goal was to observe the substrate in the active site, and we observed the product in all 3 conditions, in spite of the activity measurements. Figure 1 gives a picture of the active site with the reaction product.

Future work : We are now working on measuring long term activity on the enzyme, in order to try and find a cation with which the isomeroreductase would be inactive.

References

V. Biou, R. Dumas, C. Cohen-Addad, R. Douce, D. Job, E. Pebay-Peyroula The crystal structure of plant acetohydroxyacid isomeroreductase complexed with NADPH, two magnesium ions and a herbicidal transition state analogue, determined at 1.65Å resolution EMBO J. (1997), 16, 34053415

Dumas, R., Butikofer, M.-C., Job, D. and Douce, R. (1995) Evidence for two catalytically different magnesium binding sites in acetohydroxy acid isomeroreductase by site-directed mutagenesis. *Biochemistry, 34, 6026-6036.*