ESRF	Experiment title: **A number of Proteins from Bacteria to Eukarya and from Antarctic to Volcanic Areas**	**Experiment number**: LS-1824
Beamline: ID 14-4	**Date of experiment**: from: 23 Nov. 2000 to: 25 Nov. 2000	**Date of report**: 28/02/2001
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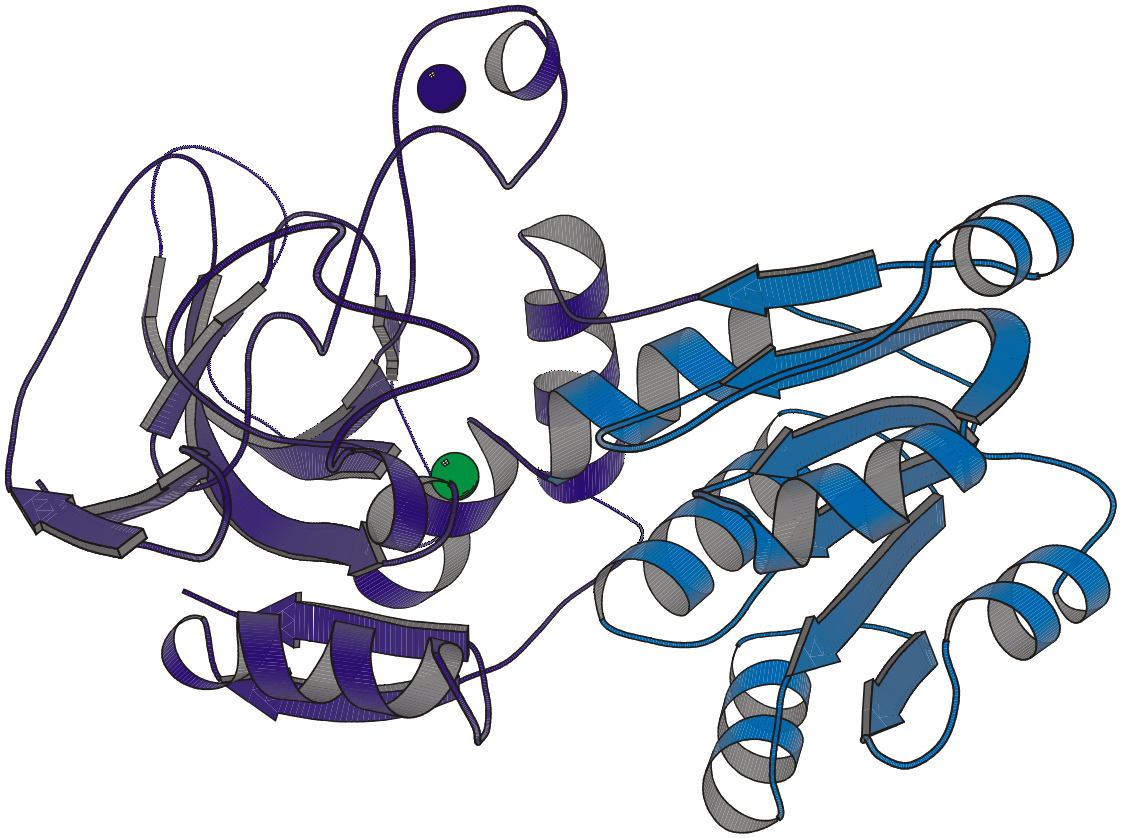
Report:

Crystal structure of alcohol dehydrogenase from the hyperthermophilic archaeon *Sulfolubus solfataricus* at 1.85 Å resolution

The structure of the alcohol dehydrogenase from the archaeon *Sulfolobus solfataricus* (SsADH)[1] has been solved by multiwavelenght anomalous diffraction, using a selenomethionine substituted enzyme. This is the first crystal structure of a medium-chain NAD(H)-dependent ADH from an archaeal hyperthermophilic organism. SsADH is a homotetrameric enzyme showing a crystallographic 222 symmetry.
Although SsADH shares a low sequence identity (about 25%) with the other mammalian or bacterial ADHs of known structure, the overall fold of its monomer is rather similar. A significant difference is the orientation of the catalytic domain relative to the coenzyme-binding domain that results in a larger inter-domain cleft.
Each monomer contains two zinc ions with a catalytic and a structural role, respectively. At the bottom of the inter-domain cleft, the catalytic zinc ion is tetrahedrally coordinated and lacks the zinc bound water molecule usually found in the apoenzyme forms. In SsADH the water is replaced by a Glu residue as found in the two bacterial ADH structures. This Glu is one of the most conserved residues in class I ADHs and it has been suggested to play a functional role in the ligand exchange at the metal centre. The new evidence of a catalytic Zn bound glutamate, provided by the SsADH structure, adds further support to the idea that a coordination of this residue to Zn could play a role in the enzymatic mechanism at least in prokaryotic dehydrogenases.
Compared to the other ADH structures, further differences are found in the architecture of the SsADH substrate pocket whose accessibility, through one of the two entrances, is more restricted.
Furthermore, SsADH is the only tetrameric alcohol dehydrogenase X-ray structure containing a second zinc ion with a structural role. This metal shows a peculiar coordination with one of the four cysteines, highly conserved throughout the structural zinc-containing dimeric ADHs, mutated to glutamic acid.

[1]*Submitted to J.Mol.Biol.*



Ribbon diagram of the SsADH monomer. The catalytic and coenzyme-binding domains are shown in blue and cyan, respectively. The catalytic Zn ion is depicted in green while the structural zinc ion is in blue.

Data collection report on SsADH MAD data (2.0 Å)

A three-wavelength MAD data set was collected on a single frozen SeMet-SsADH crystal on the ID14-4 beamline.
Based on the results coming from fluorescence measurements, diffraction data were collected to 2.0 Å resolution at wavelengths corresponding to the inflection point of the spectrum (0.9795 Å), the peak of absorption (0.9792 Å) and to a short reference wavelength (0.9537 Å).
The data were acquired on a CCD detector using 0.5° oscillations.
The data were processed with the program DENZO and SCALEPACK and a summary of the three unmerged data set statistics is shown in table 1.

Table 1: Statistics on the unmerged diffraction data

	Inflection	Peak	Reference
Wavelength (Å)	0.9795	0.9792	0.9537
Resolution (Å)	2.0	2.0	2.0
Total No. of reflections	162973	157530	163695
No. of independent reflections	58790	58628	58891
Completeness (%)	99.7 (99.9)	99.6 (99.9)	99.9 (99.9)
Rsym(I) (%)	4.6 (18.6)	4.7 (16.6)	5.0 (31.3)
Average I/σ(I)	18.8 (5.2)	16.7 (5.8)	17.2 (2.8)
f' values	-9.84	-6.79	-3.2
f'' values	3.28	6.41	4.26

Values for the outer resolution shell (2.05-2.0 Å) are given in parentheses.

Unmerged data output from SCALEPACK for all three wavelengths were input to the program SOLVE which found and refined eigth out of nine selenium sites. The well ordered sites with occupancy factors between 0.7 and 1.0 were used to phase the reflections and to produce initial maps with an initial figure of merit of 0.62 prior to density modification.

Higher resolution data, up to 1.85 Å, were also collected on a different SeMet-SsADH crystal at a pre-edge wavelength corresponding to 0.983 Å.
A data statistics summary is given in Table 2.

Table 2:

Wavelength (Å)	0.9830
Space group	$I4_122$
Cell dimensions (Å)	a=b=125.32, c=115.59
Resolution (Å)	1.85
Total No. of reflections	393222
No. of independent reflections	39225
Completeness (%)	99.9 (100.0)
Rsym(I) (%)	5.6 (35.8)
Average I/σ(I)	37.7 (6.3)

Report updated on 25 September 2001