

Experiment LS1884 - March, 30.-31., 2001

Investigation of malat dehydrogenases (MDH) from thermophilic bacteria

A total of 5 full datasets were collected during the 2 shifts allocated for this experiment. These were of two different single-site mutants of the MDH-gene from the green gliding thermophilic bacteria *Chloroflexus Aurantiacus* as well as two different metal complexes of eucaryotic ribonucleotide reductase R2-domain (R2-RNR). The mutants of C.a.-MDH are E165Q (Where glutamic acid 165 is replaced by glutamine) and T187C (where tyrosine 187 is replaced by cysteine).

The dataset of E165Q has been processed and the results presented in Table 1.

Table 1: Refinement data for C.a.-MDH mutant E165Q

Unit cell (Å)	149.87 149.87 111.40 90 90 90
Space group	P4 ₃ 2 ₁ 2
Resolution (Å)	2.25
No. reflections	47997
R-factor	24.9
R-free	28.4

The structure will be published in a paper describing the cloning, experssion, purifiacion and thermal analysis of several different tetrameric MDH-mutants. Analysis of the structure has given new insights into the thermostabilic properties of MDH from this thermophilic bacteria.

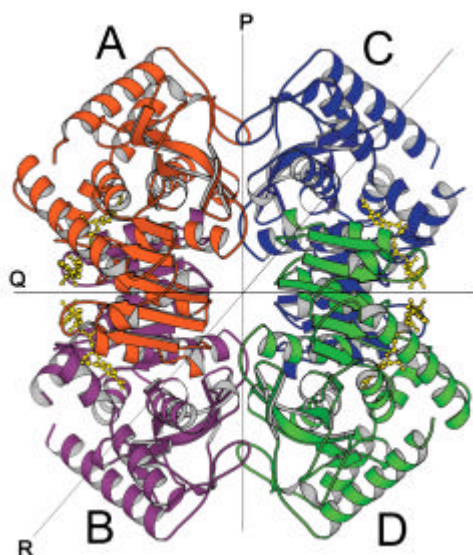


Figure 1: Overall arrangement of monomeric subunits in the bioactive tetramer.

As far as the other mutant is concerned, two datasets from two individual T187C-crystals were collected, one was the native protein crystal, the other was a crystal soaked in a Cd^{2+} solution for 3 hours. Both datasets had a maximum resolution around 2.0 Å. Unfortunately, both datasets proved to be impossible to process. We have tried various approaches with the integration software – without any success. We believe that this is due to twinning in the enantiomorphic space group $P4_32_12$. Attempts to crystallize this very interesting mutant in another space group is in progress. In fact, we believe to have managed this and new data will soon be collected.

The structures of the two metal complexes of R2-RNR have been satisfactorily refined. Selected data for the models are listed in Table 2, and the geometry of the metal center is shown in Fig. 2. A manuscript is in preparation.

Table 2: *Refinement data for Co-R2-RNR and Mn-R2-RNR*

	Co-R2-RNR	Mn-R2-RNR
Unit cell (Å)	75.1 106.9 91.5 90 90 90	75.8 107.3 92.0 90 90 90
Space group	C222 ₁	C222 ₁
Resolution (Å)	2.4	2.2
No. reflections	15274	19473
R-factor	21.8	21.1
R-free	29.7	27.8

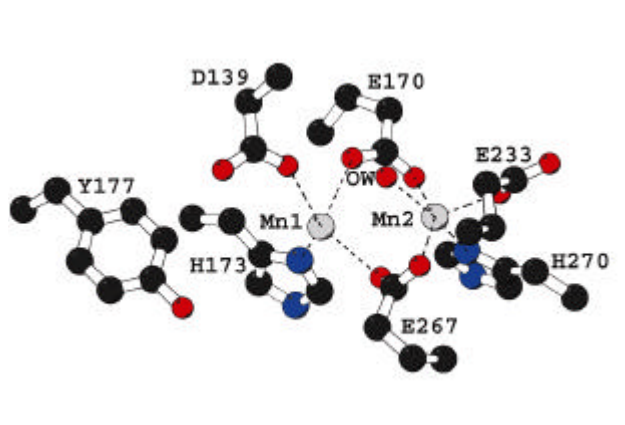


Figure 2: Metal center in Mn-R2-RNR from mouse.