Experimental report for experiment MX-159 carried out at ID14-3
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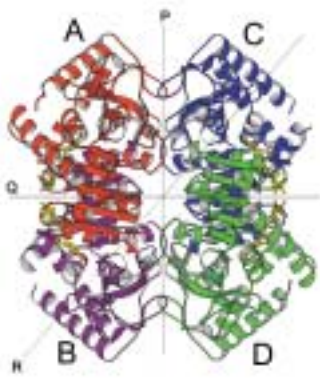
Structural basis for protein thermostability - crystal structures of thermostable tetrameric malate dehydrogenases (*)

Both from a fundamental and biotechnological point of view, there is considerable interest in thermostable enzymes. Enzymes from thermophilic organisms are often in a higher oligomeric state than their mesophilic conterparts. The relative thermal stability of tetrameric malate dehydrogenase (MDH) from *Chloroflexus aurantiacus* (*ca*-MDH), *Chlorobium tepidum* (*ct*-MDH) and *Chlorobium vibrioforme* (*cv*-MDH) can be explained by comparison of molecular interactions across the oligomeric interfaces, as revealed in the crystal structures of these proteins. [1] The contribution of the dimer-dimer interface in *ca*-MDH to thermal stability was further analyzed by studying the effects of engineering a network of electrostatic dimer-dimer interactions. Thermal stability was strongly affected by the applied mutations, with effects of single mutations on apparent melting temperature ranging from -12°C to +24°C. Thus, the oligomer stability can be drastically increased by optimization of the interaction network. This study provides one of a very few examples that underline the importance of multimerization by actually showing that, indeed, oligomeric enzymes may be dramatically stabilized by engineering the multimerization interface.

Further we have cloned and expressed several mutants of the ca-MDH that show substantially higher thermostability than the wild-type ca-MDH. In one mutant an inter-subunit disulfide bridge has been introduced in order to form a covalent link across the dimer···dimer interface, and hence stabilize the MDH tetramer [2]. In several of the mutants negatively charged residues have been replaced by either neutral or positively charged residues thus modifying the ionic network in the native ca-MDH tetramer [3]. **Using beamline ID14-3 at ESRF we have obtained diffraction data for the mutant D166N, both with and without bound metal ion in the inter-dimer region. The resolution of the best datasets are 1.55 and 1.80Å, respectively, with >99% completeness. Structures have been solved and refined [4]. In addition, a dataset for the wild-type ca-MDH without metal ion was collected for reference [3]. The resolution for this dataset was 1.9Å.**



Dataset	Dist	# frames	Exposure	Completeness	R(sym)	Resolution	I/sI
d166n_cd_1	100	200	8	> 99 %	0.05	1.55	32.6
d166n_cd_2	112	900	10	> 99%	0.06	1.60	28.6
d166n_ucd_high	136	500	10	> 99 %	0.05	1.80	37.6
d166n_ucd_low	225	180	2				
wt_ucd_high	120	750	8	> 99%	0.06	1.90	23.6
wt_ucd_low	225	225	2				

Reference

1. Dalhus, B., Sarinen, M., Sauer, U. H., Eklund, P., Johansson, K., Karlsson, A., Ramaswamy, S., Bjørk, A., Synstad, B., Naterstad, K., Sirevåg, R. & Eklund, H. J. *Mol. Biol.* 318, 707 (2002)
2. Bjørk, A., Dalhus, B., Mantzillas, D., Eijsink, V. & Sirevåg, R. *J. Mol. Biol 334, 811-821 (2003)*
3. Bjørk, A., Dalhus, B., Mantzillas, D., Eijsink, V. & Sirevåg, R. *J. Biol. Chem. To be submitted March 2004*
4. Bjørk, A., Dalhus, B., Mantzillas, D., Eijsink, V. & Sirevåg, R. *J. Mol. Biol. In preparation (2004)*

(*) In the original proposal proteins listed in this project were bacterial immunity protein EntA, an amyloid fibril precursor protein (Bence Jones) and a lipophilic insulin derivative. The experiment was originally scheduled at ID29, in the last minute we were moved to ID14-3 due to technical problems with ID29. Both the Bence Jones and insulin crystals turned out to be too small to give acceptable diffraction. We also tried to make heavy atom derivatives of entAim in order to use MIR as phasing for this project. However, no suitable metal derivative was obtained (splitting of peaks, low resolution only). (Unfortunately, we do not have a home source suitable for crystal testing of such low diffractiong samples as we are talking about here). Hence we turned to out backup project on MDH in order to maximize the use of the beam.