Crystal structure of the enamidase from *Eubacterium barkeri*

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There are several examples of bacteria that are capable of catabolizing nicotinate under aerobic conditions [1-4]. The anaerobic degradation however is by far less common in nature. Up to now only three types of bacteria are known to metabolize nicotinate without oxygen, one of them being *Eubacterium barkeri*. Parts of this unique fermentation pathway have been already resolved [5,6].

The structural characterization of enamidase is part of a study aiming on further unravelling the nicotinate fermentation pathway. Enamidase is a member of the amido-hydrolase superfamily. These enzymes catalyse the hydrolysis of a wide range of substrates bearing amide or ester groups at carbon and phosphorous centers. They contain mononuclear or binuclear metal centers embedded in a typical $(\alpha/\beta)_8$-(TIM)-barrel-structural fold.
The speciality of enamidase is its capability of catalyzing two subsequent reactions: a) the initial decyclization of 1,4,5,6-tetrahydro-6-oxonicotinate (THON) leading to 2-enamin-glutarate and b) a second hydrolysis step yielding 2S-formylglutarate (Fig. 1). There is strong evidence that the later reaction is carried out enatioselectively (unpublished data).

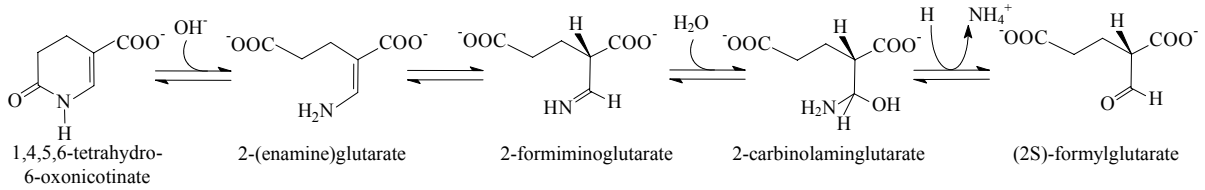

Fig. 1: The reactions catalyzed by the enzyme enamidase from *Eubacterium barkeri*

Enamidase and its selenomethionine (SeMet) derivative were expressed in *Escherichia coli* and purified via strep-tag affinity chromatography and gel-filtration. Both proteins crystallized readily under the same conditions. After flash-cooling the crystals in liquid nitrogen using glycerol as a cryo-protectant we were able to collect a native dataset at a maximum resolution of 1.9 Å. For phasing crystals of the SeMet-protein were used in a MAD experiment leading to MAD diffraction data up to 2.5 Å resolution. Both datasets were measured on the MPG/GBF beamline BW6 at DESY in January 2005.
Table 1 shows some details of the data collection and refinement statistics. Figure 2 displays the overall structure of the tetrameric enamidase with the monomers resembling the typical $(\alpha/\beta)_8$-(TIM)-barrel-structural fold of the amidohydrolase superfamily. Through X-ray fluorescence emission scans we were able to show that the binuclear metal center contains iron as well as zinc. Together with the identity and overall arrangement of the neighbouring residues this leads to the assumption that enamidase belongs to a new class of subtype II amidohydrolases [5].

In order to investigate the mechanistic details of enamidase, substrate and inhibitor co-crystallization experiments are currently under way.

	SeMEt			Wild type
A. Data collection				
Space group		C2221		C2221
Unit cell axes				
a (Å)		146.03		146.35
b (Å)		161.16		159.82
c (Å)		162.37		161.68
Wavelength (Å)	0.97943 (peak)	0.97963 (inflection)	0.95000 (remote)	1.05000
Resolution (Å)	40.4-2.5	40.4-2.5	40.3-2.5	39-1.89
Completeness (%)	100	100	100	99.1
No. reflections	244625	244157	243323	917787
No. unique reflexions	65905	65951	95898	288999
Redundancy	3.7	3.7	3.7	3.2
R_{sym} (%)	5.6 (11.6)	5.4 (11.2)	5.1 (11.9)	4.4 (25.4)
Mean I/σ I	16.0 (8.4)	16.7 (8.6)	17.5 (8.8)	18.5 (2.9)
B. Refinement				
R_{cryst} (%)				0.17
R_{free} (%)				0.20

Table 1: Data collection and refinement statistics. Values in parentheses refer to the last resolution shell

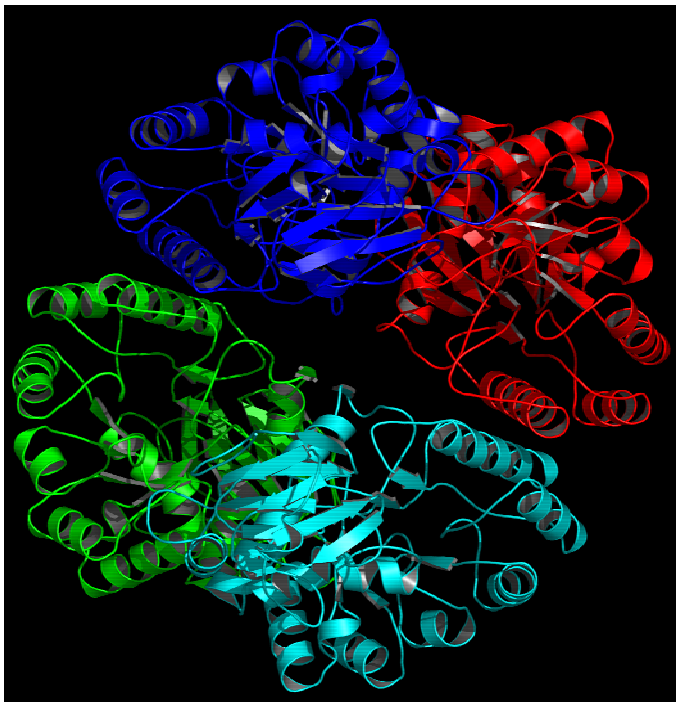

Fig. 2: Tetrameric quaternary structure of enamidase, the monomers (displayed in different colours) show the $(\alpha/\beta)_8$-(TIM)-barrel which is characteristic for the amido-hydrolase superfamily

References

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