Structure of Polyneuridine Aldehyde Esterase – an Esterase with extraordinary Substrate Specificity

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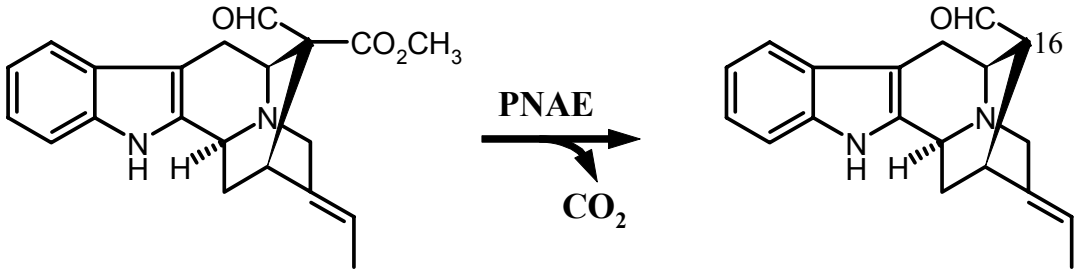
Ajmaline belongs to the monoterpenoid indole alkaloids. In the medicinal plant *Rauvolfia serpentina* (L.) BENTH. ex KURZ 10 enzymes are involved in the biosynthesis of this anti-arrhythmic drug. One of these steps is catalysed by the enzyme polyneuridine aldehyde esterase (PNAE, EC 3.1.1.78). It cleaves methanol from polyneuridine aldehyde leading to an unstable intermediate that stabilizes through non-enzymatic chiping of CO_2 and forms 16-epi-vellosimine (Figure 1). 16-epi-vellosimine is the direct precursor of the ajmaline skeleton alkaloid vinorine.



Polyneuridine aldehyde 16-epi-Vellosimine

Figure 1: Enzymatic reaction of polyneuridine aldehyde esterase (PNAE).

PNAE was heterologously expressed in *E. coli* M15 cells using the pQE-2 vector. A single IMAC-purification step with Ni-NTA was sufficient to obtain crystallisable pure enzyme. This was dialysed in 20 mM Tris-HCl, pH 7.5, 10 mM MSH, and 1 mM EDTA and the protein concentration adjusted to 3 mg/ml prior to crystalliazetion experiments.
The EMBL-HH high throughput crystallisation facility (Helgoland) was used to find the initial crystallisation conditions for the enzyme. The initial precipitant consists of 0.2 mM LiSo4, 0.1 mM BisTris, pH 6.5 and 25 % PEG 3350. After optimization the optimal crystallization condition - 0.2 M LiSO4, 0.1 M BisTris, pH 6.3 and 24 % w/v PEG 3350- was found. With this condition crystals grew in 3 days at 23 °C.
A dataset of the native enzyme with resolution up to 2.1 Å was collected at beamline X11 at the EMBL/DESY in Hamburg. The structure was solved and built *in-situ* using molecular replacement protocol of Auto-Rickshaw [2]. Within the pipeline, the program MOLREP [3], CNS [4] and automated model building program ARP/wARP [5] were used.
Formed of 264 amino acids the PNAE has a calculated molecular weight of 29.65 kDa. High sequence homology places PNAE in the alpha/beta hydrolase family, this was confirmed after structure elucidation (Figure 2). The usual catalytic triad of alpha/beta hydrolase is formed in PNAE with the residues Ser87, Asp216 and His244, as proven by site-directed mutagenesis [1]. In contrast to many other esterases, PNAE is very substrate-specific. In order to gain detailed knowledge on the mechanism of this specific esterase, the knock-out mutant His244Ala was

prepared and crystallized. Its structure was determined to a resolution of 3.1A. A resolution of 2.2A could be obtained at the SLS in Villigen. A complex with the substrate is in progress.

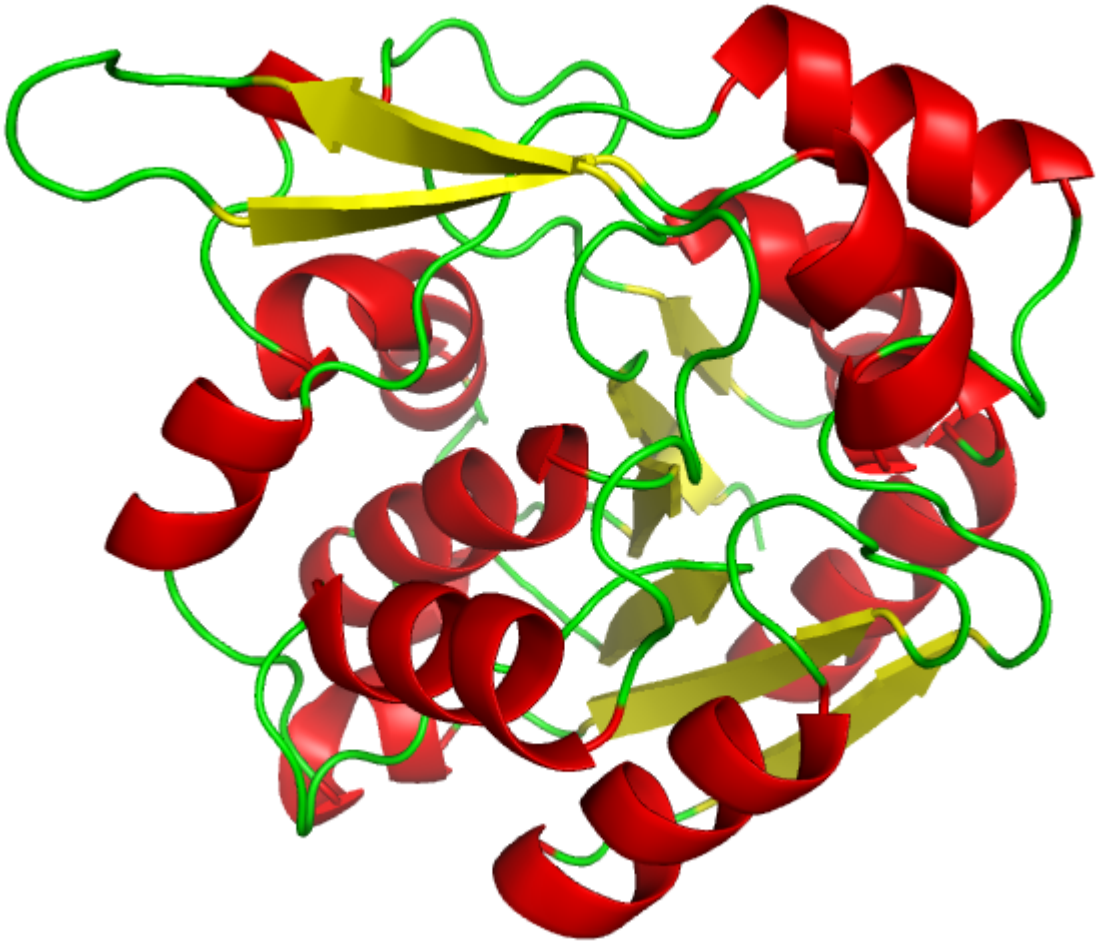


Figure 2: Cartoon representation of polyneuridine aldehyde esterase (PNAE).

References

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