

Elucidating the catalytic mechanism of cysteine dependent amidases and nitrilases

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Of considerable interest are the determinants of nitrilase activity, in which a nitrile is converted to ammonia and the corresponding carboxylic acid, versus amidase activity, in which an amide is converted to ammonia and the corresponding carboxylic acid. In both cases it appears that the main catalytic residues are a cysteine, two glutamates and a lysine. Three recombinantly expressed archaeal enzymes of the nitrilase superfamily have been purified and characterized. The PaNit from *Pyrococcus abyssi* has been reported as true nitrilase which prefers dinitrile substrates. Its X-ray structure (PDB ID 3IVZ) shows an almost identical active site to that of the known amidases. The AmiE from *Pyrococcus yanosii* shares 78% sequence homology with PaNit has an amidase activity, hydrolyzing acetamide, L-glutamine, hexanamide, acrylamide, formamide and L-asparagine but no nitrilase activity against the corresponding nitriles. A similar enzyme found in *Pyrococcus horikoshii* was crystallized and its structure was determined (PDB ID 1J31) to be extremely similar to PaNit (RMSD = 0.45Å and the sequence identity is 86%). However, no functional characterization of this enzyme was reported.

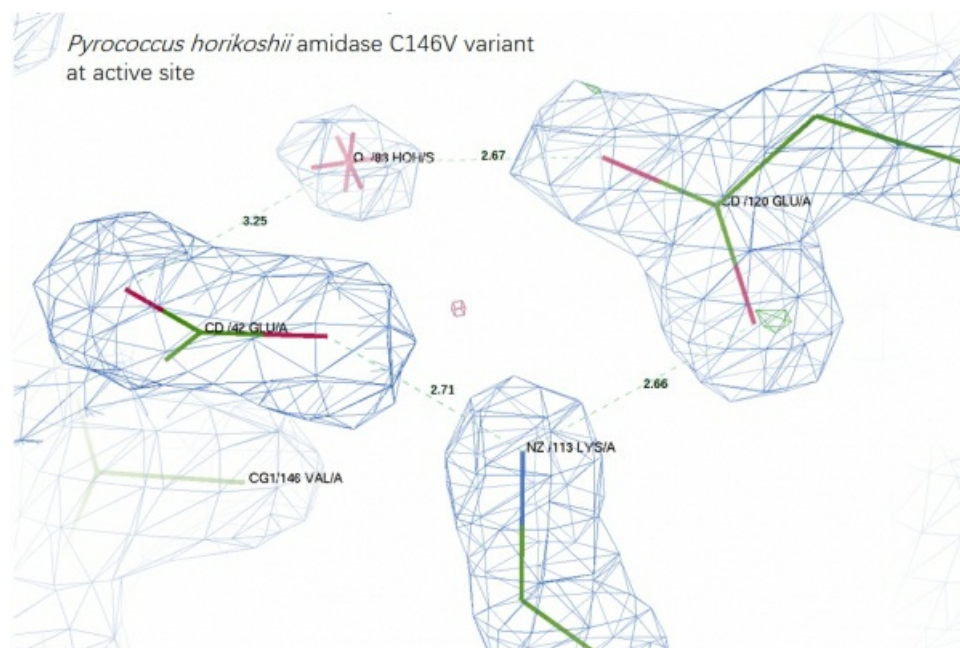
The genes from the archaeal enzymes from *P. abyssi* and *P. horikoshii* were synthesized and were inserted in expression plasmids that contain a C-terminal, thrombin cleavable his-tag. Abundant soluble expression of both proteins was obtained from the constructs. Amidase activity was observed for formamide, acetamide, propionamide, butyramide, hexanamide and malonamide but no nitrilase activity was observed for any substrate.

The crystal structure of C146V variant of the enzyme from *P. horikoshii* has been determined and will be used to observe substrate binding in the active site.

[1] Fu, L. et al. (2014). *Extremophiles*. 18, 429–440.

[2] Mueller, P. et al. (2006). *Protein Expr Purif*. 47, 672–681.

[3] Nel et al. (2011). *Appl. Environ. Microbiol*. 77, 3696–3702.



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