Crystal Structure of Nitrile Hydratases: Possible Industrial Usage

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Nitrile hydratase (NHase) is a metalloenzyme that catalyzes hydration of nitriles to corresponding amides. It is of major interest because of its use for synthesising industrial products such as acrylamide and nicotinamide [1]. NHases typically consist of two subunits (α and β) with similar molecular masses (23 and 25 kDa) and either a single non-heme Fe^{III} or non-corrinoid Co^{III} per αβ dimer [1].

We have purified, crystallised and determined the structure of wild type (WT) and mutant NHases from Bacillus RAPc8. The space group was determined to be primitive tetragonal (p41212). The WT structure was solved at 2.1Å using molecular replacement (MR) with a 65% homologue from *P. thermophila*.

The 2.5-3.0Å data from isomorphous crystals of F36L, F52L, F55L, Y67A and W76G mutants were solved by MR using the WT structure. An interesting result came from the F55L mutant map, showing apparent flexibility of F52. The flexible F52 might be related to substrate access to the active site. In order to understand this we are doing activity tests for the F55L mutant. The other interesting result came from normal mode analysis about the flexibility of the protein. Our aim is to combine structural data with biochemical results to understand the mechanism of this enzyme better, and so search for possible improvement in activity for industrial biotransformation.

[1] Cowan D.A., Cameron R.A., TsekoaL.T., *Advances in Applied Microbiology*, 2003, **52**, 123.

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