Refinement of the Structures of WT and Mutant Nitrile Hydratases from *Bacillus* RAPc8

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Nitrile hydratase (NHase) is a metalloenzyme that catalyzes hydration of nitriles to corresponding amides. This enzyme is of major interest to industry and academia because of its use for synthesising industrial products such as acrylamide and nicotinamide (reviewed in 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 $\alpha\beta$ dimer (1).

The structure was solved at 2.5$\mathring{A}$ using molecular replacement with 65% homologue from *Pseudonocardia thermophila*. This gave a map in which the backbone in the homologous region was generally well resolved. Side chain substitution using SCWRL dramatically improved the quality of the phasing and clearly revealed density that was previously invisible. Approximately 50 residues were built *de novo* into this density using O.

The 3.0$\mathring{A}$ data from isomorphous crystals of the inactive F55L mutant was solved by molecular replacement using the WT structure. An unexpected difference in the mutant map was the apparent flexibility of F52. Both F55 and F52 are around a channel leading to the active site. The inactivity of the mutant may be explained by the flexible F52 inhibiting substrate access to the active site. Crystals of other mutants are ready for analyses. Our aim is to use information from the crystal structures to generate genetically engineered NHases with improved performance in industry.

[1] Cowan, D.A., Cameron, R.A. and Tsekoa, L.T. (2003) Comparative Biology of Mesophilic and Thermophilic Nitrile Hydratases. Advances in Applied Microbiology 52, 123-158.