Crystallisation and Structural Studies on

Omega Transaminase Enzymes

Submitted by
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**Abstract**
Omega transaminase enzymes are proving to be of vital importance in the production of chiral amines for drug manufacturing due to their high stereoselectivity and regioselectivity reactions. Omega transaminases also possess another key feature that can make them more desirable than other transaminase enzymes in that they are able to utilise substrates lacking a carboxyl group.

This project has purified several recombinant omega transaminases from the bacteria; *Streptomyces avermitilis*, *Deinococcus geothermalis*, *Pseudomonas putida* and *Pseudomonas aeruginosa*. Attempts have been made to crystallise the proteins in order to determine their structures. *D. geothermalis* omega transaminase failed to purify due to lack of binding to Nickel columns despite having a histidine tag. *P. putida* omega transaminase failed to produce any crystals in the crystal screens available. *P. aeruginosa* omega transaminase crystals, whose holoenzyme structure has already been solved, were soaked in pyruvate and also produced crystals from co-crystallisation experiments with pyruvate. These crystals however did not show sufficient electron density in the active site to determine if the substrate had bound. They did however diffract to 2.0 Å and 1.8 Å resolution respectively. Co-crystallisation experiments with *P. aeruginosa* omega transaminase and the inhibitor gabaculine were successful however to date they have failed to produce X-ray diffraction data that could be processed despite diffracting to high resolution.

The *S. avermitilis* omega transaminase produced crystals that were shown to be disordered when they underwent X-ray diffraction. Differential scanning fluorometry was carried out to identify the optimal buffer for protein stability. This identified the current Tris pH 7.5 buffer to lead to a low thermodynamic stability of the protein. Several new buffers including MES at pH 6 were identified as being the most likely candidates for stabilising the protein and producing ordered crystals.

Data collected from a *Chromobacterium violaceum* omega transaminase with a F89 to A89 mutation was successfully processed and refined. The resultant structure showed an increase in flexibility in the substrate carboxyl binding residue, R416, and an increase in space available for substrate binding. HPLC assays showed alpha-methylbenzylamine activity remained. An amino acid oxidase/horseradish peroxidase linked assay showed there to be a small increase in activity towards β-alanine due to this mutation. Further mutations will be necessary to enable this enzyme to utilise β-alanine at a more practical rate of conversion.
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