The purification and crystallisation of 2,5-diketocamphane 1,2 monoxygenase and 3,6 diketocamphane 1,6 monoxygenase from Pseudomonas putida NCIMB 10007

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It is known that Pseudomonas putida NCIMB 10007 is equally able to grow on either enantiomer of camphor as the sole carbon source [1,2]. These reports have shown that the metabolism of (+)-camphor results initially in the formation of a 5-exo-hydroxycamphor intermediate. This is subsequently oxidised to form 2,5-diketocamphane, which then undergoes a Baeyer-Villager oxidation, therefore forming a lactone.

Later studies have indicated that the (-)-camphor undergoes an independent metabolism [3], with the metabolites in this case being mirror images from the (+) pathway. The enzymes involved are also separately induced (Figure 1).

The monooxygenases that effect the Baeyer-Villager reaction of the enantiomeric 2,5- and 3,6-diketocamphane intermediates from the (+) and (-) pathways respectively, have been found to have a trimeric structure, composed of oxygenating subunits linked loosely to an NADH dehydrogenase. These subunits are thought to interact through the cofactor FMN [4]. Previous work has described some purification of these subunits [5,6].

Both isozymes have been found to carry out a number of chemical biotransformations [7-10]. High yields and optical purity have been obtained using a variety of natural ketones and their analogues. The isozymes possess distinct and useful enantioselective properties.

The monooxygenase protein has been grown in 0.2M CaCl2, 0.1M sodium acetate. Both crystal forms show diffraction and are suitable for X-ray analysis. The latter crystal was found to diffract to beyond 3.5Å resolution when using Synchrotron radiation at Daresbury Laboratories, UK.

This report has described a rapid and reproducible method for the purification of the NADH dehydrogenase subunit was achieved by purifying the dialysed 50% ammonium sulfate pellet remaining from the initial purification stage [11] using Fast flow-Q™ anion-exchange chromatography. This was followed by dye-ligand affinity chromatography using Blue B (Amicon). Protein resulting ran as one band on SDS-PAGE, with an estimated molecular weight of 36,000Da. Crystal clusters of the 2,5-oxygenating subunit have been grown in 0.2M CuCl2, 0.1M HEPES and 30% PEG 400.

Plate shaped crystals of the 3,6-oxygenating subunit have been grown in both 4.0M sodium formate and 28% PEG 8000 in 0.2M sodium acetate. Both crystal forms show diffraction and are suitable for X-ray analysis. The latter crystal was found to diffract to beyond 3.5Å resolution when using Synchrotron radiation at Daresbury Laboratories, UK.

This report has described a rapid and reproducible method for the purification of the 2,5- and 3,6-oxygenating subunits, as well as their NADH dehydrogenase subunit. Further information on the structure of these enzymes obtained from the diffraction of the crystals will expand their use in biotransformation reactions.

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Abbreviations used: FMN= Flavin mononucleotide