BIOCHEMICAL AND STRUCTURAL CHARACTERISATION OF DEHALOGENASES FROM MARINE BACTERIA

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Abstract

An L-haloacid dehalohenase from the psychrophilic marine bacteria *Psychromonas ingrahamii* has been cloned, over-expressed in a bacterial expression system and biochemically characterised. The enzyme is stable at temperatures of up to 60ºC for 90 min and shows highest activity towards substrates with short carbon chains (≤C3). The enzyme is stable in up to 30% ethanol, methanol and DMSO when incubated for 1 h. The *Km* for the enzyme is 1.36 mM.

The genome of AQP5750 from the Aquapharm Biodiscovery Ltd Microbial library was sequenced. An L-haloacid dehalohenase and a haloalkane dehalogenase gene were identified within the genome.

The AQP5750 L-haloacid dehalohenase has been cloned, over-expressed in a bacterial expression system, biochemically characterised, crystallized and the native and crystal complex structure with chloropropionic acid (MCP) determined. The L-haloacid dehalohenase from AQP5750 shows highest activity at 55ºC towards brominated substrates with short carbon chains (≤C3). The enzyme shows increased activity of 150% in 40% DMSO and 123% in 30% methanol. The L-haloacid dehalohenase crystal complex structure with covalently bound MCP confirmed Asp 18 as the main catalytic residue. Residues His 183, Asp 186 and Glu 21 in the active site are proposed to be involved in activation of the catalytic water which attacks the ester intermediate in the second part of the SN2 dehalogenase mechanism.

The AQP5750 haloalkane dehalogenase has been cloned, over-expressed in a bacterial expression system, crystallized and the native and complex structure with 1-hexanol has been determined. Substrate specificity experiments showed that the haloalkane dehalogenase from AQP5750 does not show high activity towards substrates used by other haloalkane dehalogenases with high amino acid sequence identity. The large active site cavity and the presence of Ser 176 and Arg 136 in the hydrophobic binding pocket may alter the binding of substrates tested which could account for the low activity observed.

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