EXPRESSION, PURIFICATION AND MUTAGENESIS OF RECOMBINANT CLASS 1 ALDEHYDE DEHYDROGENASE

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ABSTRACT

Aldehyde dehydrogenase (AlDH) catalyses the conversion of aldehydes, for example acetaldehyde and retinal, to carboxylic acids in an NAD^+-dependent reaction involved in the detoxification of aldehydes and alcohols. There are several isoenzymes, class 1 being the cytosolic form.

Three over-expression and purification systems have been tested, in order to gain a high yield of active, pure class 1 aldehyde dehydrogenase. The traditional method, using T7 polymerase-driven expression in *E. coli* followed by ion-exchange and *p*-hydroxyacetophenone-affinity chromatography, gave 3 mg/L human aldehyde dehydrogenase with a high specific activity of 1.2 units/mg. Human Class 1 AlDH has been over-expressed and purified using the GST Gene Fusion System (Pharmacia Biotech), avoiding the need for AlDH-affinity chromatography and therefore allowing straight-forward purification of mutated enzymes. The GST fusion system produced 2.6 mg/L pure AlDH with a specific activity of 0.39 units/mg. The methylotrophic yeast *Pichia pastoris* was chosen for its high yields, in the region of grams/litre. Preparatory work was carried out with the construction of the expression plasmids and screening of the *Pichia* transformants.

A highly conserved lysine residue (Lys-272) may be involved in acid-base catalysis of aldehyde oxidation, as well as of the esterase reaction also catalysed by AlDH. Preliminary work has been carried out on the generation of the K272A, K272R, K272H and K272L altered enzymes. The resultant activity level, kinetic behaviour and active site structure of these modified enzymes should help to elucidate further the mechanism of action of AlDH.

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