

P010 Cloning, expression and characterisation of *Streptomyces coelicolor* xanthine dehydrogenase, and the role of XdhC in the enzyme maturation process

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Xanthine dehydrogenase (XDH) enzymes are complex, metallo-flavoproteins catalysing the oxidative hydroxylation of purines, pterins and aldehyde substrates. They contain three redox active components, 2x [2Fe-2S] clusters, FAD and the Mo-cofactor. The *xdhABC* operon from the Gram-positive bacterium *Streptomyces coelicolor* was cloned and expressed in the *E.coli* TP1000 strain, which accumulates the required unmodified Mo-cofactor, to produce active XDH enzyme. The protein has been purified and exhibits typical characteristics and activities of XDH. Steady-state kinetic analysis also reveals weak, positive cooperativity during oxidation of xanthine, its primary substrate. XDH expressed in the absence of XdhC is 'immature' and inactive. XdhC has been purified in a soluble form and some of its properties determined for the first time. XdhC interacts *in vitro* with both active and 'immature' forms of XDH, using the purified components, while crude extracts of 'immature' XDH can be partially activated using crude extracts containing XdhC or purified XdhC. These results highlight the complexity of the XDH-specific maturation machinery.