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Histidinol dehydrogenase from Neurospora crassa

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Abstract

Histidinol dehydrogenase from Neurospora crassa

Creaser, E.H. and R. B. Drysdale. Histidinol dehydrogenase from Neurospora crassa.

It is thought that the locus histidine-3 controls two functions in Neurospora, the first of these being in the early stages of histidine biosynthesis and largely unknown at present. The second function is to direct the formation of the terminal enzyme in the sequencehistidinol dehydrogenase. We have studied the purification and some properties of this enzyme. The enzyme can be extracted from wet mycelium by grinding with glass powder or from dried mycelium by extraction with pH 9.1 Tris buffer. The extract is treated with 0.05M MnCl₂ to precipitate nucleic acids and unwanted proteins. Ammonium sulphate is added to 50% saturation and the precipitate discarded. The saturation is increased to 65% and the precipitate which contains the enzyme is retained. The enzyme is quite heat stable and can be further purified by heat treatment and chromatography on DEAE cellulose. The product accounts for approximately 0.1% of the protein of Neurospora. The enzyme is specific for NAD, its optimum pH is in excess of 10, it is Mg⁺⁺ activated and aged preparations can be reactivated by thioglycollic acid or cysteine. Normal and sucrose density gradient ultracentrifugation indicates a molecular weight of 36,000 to 40,000. The enzyme from heterocaryons and also from histidine-3 mutants which lack the first function can be purified by the same procedures.---Department of Microbiology, University of

Birmingham, Birmingham, England.