PURIFICATION AND PROPERTIES OF URACIL DEHYDROGENASE FROM *Nocardia corallina*

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ABSTRACT

Uracil dehydrogenase (EC 1.2.99.1), an enzyme which catalysed the oxidation of uracil or thymine to the corresponding barbituric acids, has been purified in a typical preparation, 85-fold with an 18% recovery of the initial activity present in a cell-free extract from Nocardia corallina. The enzyme was considered to be 95-98% pure by gel electrophoresis. It has a molecular weight of approximately 298 000, determined by both gel filtration and sedimentation equilibrium centrifugation and consists of two each of three dissimilar subunits, with molecular weights of 91 000, 36 000 and 21 000 as determined by sodium dodecyl sulphate — polyacrylamide gel electrophoresis. The spectrum of the enzyme is similar to that of milk xanthine oxidase and rabbit liver aldehyde oxidase (Rajagopalan & Handler, 1964), and is typical of nonheme iron flavoproteins. The flavin prosthetic group was identified as flavin adenine dinucleotide. It was demonstrated that the enzyme contains, per mole of protein, 1 mole of FAD, 4 atoms of nonheme iron and 4 moles of labile sulphide. The amino acid composition of uracil dehydrogenase has been determined. Other properties reported for the enzyme include substrate and electron acceptor specificity, K_m for uracil and thymine, pH optimum, and the effect of various inhibitors on enzyme activity. In addition, the purified enzyme has been shown to exhibit 'aerobic dehydrogenase' activity.

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