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Microbial Metabolism of Quinoline and Related Compounds

XX. Quinaldic Acid 4-Oxidoreductase from *Pseudomonas sp.* AK-2 Compared to other Procaryotic Molybdenum-Containing Hydroxylases

Monika SAUTER, Barbara TSHISUAKA, Susanne FETZNER and Franz LINGENS

Institut für Mikrobiologie der Universität Hohenheim

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Dedicated to Prof. Dr. H. Hellmann on the occasion of his 80th birthday

Summary: Quinaldic acid 4-oxidoreductase from *Pseudomonas sp.* AK-2 catalyses the hydroxylation of quinoline 2-carboxylic acid (quinaldic acid) to 4-hydroxyquinoline 2-carboxylic acid (kynurenic acid) with concomitant reduction of a suitable electron acceptor. An analogous hydroxylation in *para*-position relative to the N-heteroatom was only recently described for quinaldine 4-oxidoreductase (de Beyer & Lingens, 1993, *Biol. Chem. Hoppe-Seyler* **374**, 101–110) and for quinaldic acid 4-oxidoreductase from *Serratia marcescens* 2CC-1 (Fetzner & Lingens, 1993, *Biol. Chem. Hoppe-Seyler* **374**, 363–376).

Quinaldic acid 4-oxidoreductase from *Pseudomonas putida* AK-2 was purified 78-fold to electrophoretic homogeneity with a recovery of 22%. The native enzyme (300 kDa) was composed of three subunits with molecular masses of 90, 34 and 20 kDa, indicating an $\alpha_2\beta_2\gamma_2$ structure. Quinaldic acid 4-oxidoreductase contained FAD, molybdenum, iron and acid-labile sulfur in a ratio of 2:2:8:8. Molybdenum is probably associated with molybdopterin cytosine dinucleotide as organic part of the pterin molybdenum cofactor. The absorption spectrum of quinaldic acid 4-oxidoreductase exhibited the typical features of a molybdo-iron/sulfur-flavoprotein, namely, maxima at 274 nm, 340 nm and 450 nm, a shoulder at 550 nm, a ratio A_{280}/A_{450} of 4.7 and a ratio A_{450}/A_{550} of 3.5.

The enzyme was susceptible to inactivation by methanol, sodium *m*-arsenite, *p*-hydroxymercuribenzoate, and potassium cyanide. Cyanide caused an alteration at 320 nm in the absorption spectrum, typical for the change in the coordination sphere of the molybdenum. Enzyme inactivated with cyanide was reactivated to 74% by incubation with sulfide. Thus, quinaldic acid 4-oxidoreductase possesses a monooxo-monosulfido-type molybdenum center.

Key terms: Quinaldic acid 4-oxidoreductase, molybdenum-containing hydroxylase, pterin molybdenum cofactor, molybdopterin cytosine dinucleotide, molybdo-iron/sulfur-flavoprotein, *Pseudomonas sp.* AK-2.

Enzymes:
Carbon monoxide dehydrogenase, carbon monoxide:(acceptor) oxidoreductase, (EC 1.2.99.2);
6-Hydroxynicotinate dehydrogenase, 6-hydroxypyridine 3-carboxylic acid:(acceptor) 2-oxidoreductase (hydroxylating);
Isoquinoline 1-oxidoreductase, isoquinoline:(acceptor) 1-oxidoreductase;
Nicotinate dehydrogenase, pyridine 3-carboxylic acid:(acceptor) 6-oxidoreductase (hydroxylating), (EC 1.5.1.13);
Nicotine dehydrogenase, nicotine:(acceptor) 6-oxidoreductase (hydroxylating), (EC 1.5.99.4);
Quinaldic acid 4-oxidoreductase, quinoline 2-carboxylic acid:(acceptor) 4-oxidoreductase;
Quinaldine 4-oxidoreductase, 2-methylquinoline:(acceptor) 4-oxidoreductase;
Quinoline 2-oxidoreductase, quinoline:(acceptor) 2-oxidoreductase;
Xanthine oxidase, xanthine:(oxygen) oxidoreductase, (EC 1.2.3.2).

Abbreviations:
AMP, adenosine 5′-monophosphate; CMP, cytidine 5′-monophosphate; EDTA, ethylenediaminetetraacetic acid; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; FPLC, fast protein liquid chromatography; GMP, guanosine 5′-monophosphate; INT, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride; MCD, molybdopterin cytosine dinucleotide; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; UMP, uridine 5′-monophosphate.