Biol. Chem. Hoppe-Seyler
Vol. 374, pp. 363–376, June 1993

Microbial Metabolism of Quinoline and Related Compounds

XVIII. Purification and Some Properties of the Molybdenum- and Iron-Containing Quinaldic acid 4-oxidoreductase from *Serratia marcescens* 2CC-1

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(Received 27 April 1993)

Summary: *Serratia marcescens* 2CC-1 utilizes quinaldic acid (quinoline 2-carboxylic acid) as sole source of carbon, nitrogen and energy. Growth of strain 2CC-1 on quinaldic acid as well as on nicotinic acid and hypoxanthine was inhibited completely by the molybdate antagonist tungstate, whereas growth on kynurenic acid and 6-hydroxynicotinic acid was not affected by tungstate. The synthesis of the molybdenum-containing hydroxylases quinaldic acid 4-oxidoreductase and nicotinic acid 6-oxidoreductase was found to be inducible. In addition, *Serratia marcescens* 2CC-1 produced a constitutively expressed xanthine oxidoreductase.

Quinaldic acid 4-oxidoreductase was purified 1075-fold with a recovery of 5%. For catalytic activity, artificial electron acceptors were necessary. The 95–100-kDa enzyme was a heterodimer with subunit molecular masses of 75–80 kDa and 18–19 kDa. Quinaldic acid 4-oxidoreductase contained 2.3–3.7 g atom of iron and 0.5–0.6 g atom of molybdenum per mol of enzyme. The absorption spectrum exhibited maxima at 280 nm, 334 nm, 480 nm and a shoulder at 550 nm, with A_{280}/A_{334} = 4.8, A_{280}/A_{450} = 10.0, A_{280}/A_{480} = 9.4, and A_{450}/A_{550} = 1.6, suggesting the absence of a flavin cofactor.

Acridine, quinacrine, ethylenediaminetetraacetate, 2,2′-dipyridyl, 1,10-phenanthroline and iodoacetate did not affect enzyme activity. *p*-Hydroxymercuribenzoate, *m*-arsenite, cyanide and methanol were effective inhibitors of quinaldic acid 4-oxidoreductase. Cyanide-inhibited enzyme was reactivated by treatment with $S^{2\ominus}$, indicating the presence of a pterin molybdenum cofactor with a monooxo-monosulfido-type molybdenum center. Quinaldic acid 4-oxidoreductase showed a very high substrate specificity, quinaldic acid being the only substrate found to be transformed significantly.

Enzymes:
Aldehyde oxidase, aldehyde:oxygen oxidoreductase (EC 1.2.3.1);
Arsenite oxidase, arsenite:oxygen oxidoreductase;
Carbon monoxide dehydrogenase, carbon monoxide:(acceptor) oxidoreductase (EC 1.2.99.2);
Dihydroorotase, L-5,6-dihydro-orotate amidohydrolase (EC 3.5.2.3);
6-Hydroxynicotinic acid dehydrogenase, 6-hydroxypyridine 3-carboxylic acid:(acceptor) 2-oxidoreductase (hydroxylating);
Nicotine dehydrogenase, nicotine:(acceptor) 6-oxidoreductase (hydroxylating), EC 1.5.99.4;
Nicotinic acid dehydrogenase, pyridine 3-carboxylic acid:(acceptor) 6-oxidoreductase (hydroxylating), EC 1.5.1.13;
Quinaldic acid 4-oxidoreductase, quinoline 2-carboxylic acid:(acceptor) 4-oxidoreductase (hydroxylating);
Quinaldine 4-oxidoreductase, 2-methylquinoline:(acceptor) 4-oxidoreductase (hydroxylating);
Quinoline 2-oxidoreductase, quinoline:(acceptor) 2-oxidoreductase (hydroxylating);
Quinoline 4-carboxylic acid 2-oxidoreductase, quinoline 4-carboxylic acid:(acceptor) 2-oxidoreductase (hydroxylating);
Sulfite oxidase, sulfite:oxygen oxidoreductase, EC 1.8.3.1;
Xanthine dehydrogenase, xanthine:$NAD^{\oplus}$ oxidoreductase, EC 1.1.1.204;
Xanthine oxidase, xanthine:oxygen oxidoreductase, EC 1.1.3.22.

Abbreviations:
EDTA: ethylenediaminetetraacetate; FPLC: fast protein liquid chromatography; INT: 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride; MCD: molybdopterin cytosine dinucleotide; MPT: molybdopterin; PAGE: polyacrylamide gel electrophoresis; PMS: *N*-methyldibenzylpyrazine methylsulfate; PVDF: polyvinylidene difluoride; SDS: sodium dodecyl sulfate.