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Cell Free Enzyme Studies on Naphthol Reductase from *Verticillium dahliae*

by

Matara K. B. Weerasooriya

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School of Chemistry
University of Bristol
Cantock's Close
BS8 1TS

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Abstract

Rice blast disease, caused by fungus *Pyricularia oryzae*, is a serious problem in temperate climates. To date the most effective anti-blast agents are the antipenetrants such as tricyclazole, pyroquilon and PP-389. These compounds have been shown to inhibit the biosynthesis of melanin, which is essential for pathogenicity. The biosynthetic pathway of 1,8-DHN (1,8-dihydroxynaphthalene) melanin in *P.oryzae* is well documented and the evidence for the correlation between loss of pigmentation and the loss of pathogenicity is discussed. The mechanisms by which blocking melanin biosynthesis leads to failure of appressorial penetration remains unresolved but several possible explanations are presented.

The preparation of substrates for studies on the isolation of the key reductase enzyme on the melanin biosynthetic pathway was investigated. Unlabelled 1,3,6,8-tetrahydroxynaphthalene and 1,3,8-trihydroxynaphthalene were synthesized and scytalone and vermelone were isolated from *Phialaphora largerbergii* and *Verticillium dahliae brm-1* cultures. Initial attempts to introduce ^{14}C and ^{3}H labels into 1,3,6,8-tetrahydroxynaphthalene proved that both ^{14}C and ^{3}H labelled forms of the compound are not suitable substrates for the purification of the reductase. It was shown that the synthesis of ^{3}H labelled 1,3,8-trihydroxynaphthalene is practicable, but studies showed that due to the levels of the substrate required, it is also not an ideal substrate. Finally HPLC techniques were developed to analyse the conversion of unlabelled 1,3,6,8-tetrahydroxynaphthalene to scytalone as a satisfactory assay for the purification of the enzyme.

The NADPH dependent naphthol reductase of the mutant *V. dahliae brm-1* was isolated and partially purified using ion exchange and gel-filtration chromatography. The molecular weight of the enzyme found to be ~ 24,000 Da. Kinetic behaviour, primary sequence, substrate specificity and inhibitors of the enzyme were investigated.

Deuterium labels incorporated at the 2- and 4-positions of 1,3,8-trihydroxynaphthalene were found to be highly labile. Incubation studies of 1,3,8-trihydroxynaphthalene in deuterated buffer with and without reductase proved that reduction is linked to the availability of keto-phenol tautomers of 1,3,8-trihydroxynaphthalene. The presence of these tautomers and the proton exchange rates of these forms at pH 6, 7 and 8 were studied. Even though the predominant existence of keto form of 1,3,8-trihydroxynaphthalene was observed at pH 9, *in vitro* reduction of 1,3,8-trihydroxynaphthalene under this condition using sodium borohydride failed.