the corresponding spectra of adrenodoxin. Thus according to our interpretation of the spectra both of these hydroxylase iron-sulphur proteins have a similar mode of action to the plant ferredoxins.

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Some Studies on the Purified 2-Furoyl-Coenzyme A Hydroxylase from *Pseudomonas putida* F2

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The direct conversion of the carbon skeleton of furan-2-carboxylate (2-furoate) into 2-oxoglutarate by *Pseudomonas putida* F2 has been demonstrated by Jones & Trudgill (1967). The sequence of intermediates furan-2-carboxylate $\rightarrow$ 2-furoyl-CoA $\rightarrow$ 5-hydroxy-2-furoyl-CoA $\rightarrow$ 5-oxo- Δ^2 -dihydro-furoyl-CoA $\rightarrow$ 2-oxoglutaryl-CoA $\rightarrow$ 2-oxoglutarate has been proposed, and the 2-furoyl-CoA hydroxylase has been separated from 2-furoyl-CoA synthetase and the other enzymes of the sequence (Trudgill, 1969; Kitcher & Trudgill, 1970).

The 2-furoyl-CoA hydroxylase draws its oxygen from water and, like other enzymes that catalyse analogous hydroxylations of nicotinate (Hunt, Hughes & Lowenstein, 1958) and picolinate (Dagley & Johnson, 1963), has an obligatory requirement for an electron acceptor. This requirement may be satisfied by Methylene Blue, Nitro Blue Tetrazolium or the membrane fraction from furan-2-carboxylate-grown *Ps. putida* F2. Measurement of the reduction of the artificial electron acceptor under suitable conditions or coupling of Methylene Blue reduction to oxygen consumption provide acceptable methods for detection and assay of the enzyme.

The 2-furoyl-CoA hydroxylase has been purified about 100-fold by ammonium sulphate fractionation (0–30% saturation fraction), chromatography on Agarose A5-m and sucrose-density-gradient centrifugation. Sufficient purified enzyme has not yet been obtained for an assessment to be made of its degree of purity. Its high molecular weight, about 900 000, estimated by comparison with marker

proteins on the agarose column and sucrose density gradients, precludes the use of such micro techniques as isoelectric focusing and polyacrylamide-gel electrophoresis on the native protein.

On spectral analysis the enzyme displays a single absorption peak with $\lambda_{\max}$ 273nm. This observation, coupled with the lack of significant inhibition by metal-ion-chelating agents, implies that this hydroxylase, in contrast with those that utilize molecular oxygen directly, contains neither metal ion nor flavin cofactors (Hayaishi, 1969).

The purified enzyme displays K_m 2×10^{-5} M for 2-furoyl-CoA. In the presence of an electron acceptor the purified enzyme catalyses the formation of a product that displays an absorption maximum at 373nm. Addition of crude extract, which contains the enzymes required to convert the hydroxylation product into 2-oxoglutarate (Kitcher & Trudgill, 1970), catalyses a time-dependent decrease in the 373nm extinction.

On the basis of a comparison with the spectral properties of 2-carbethoxy-5-hydroxythiophen it is proposed that the reaction product showing $\lambda_{\max}$ 373nm is 5-oxo- Δ^7 -dihydro-2-furoyl-CoA.

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Hydroxylation and Toxicity of Aflatoxins

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There are at least four routes for the biological hydroxylation of this group of potent hepatotoxins: (1) a hydroxyl group may be inserted at the junction of the two fused furan rings, e.g. aflatoxin B₁ is converted into aflatoxin M₁; (2) the single methoxyl group in the aromatic portion of the molecule may be hydrolysed to form a phenolic derivative; (3) the vinyl ether double bond of aflatoxins B₁ and G₁ may be hydrated, yielding aflatoxin hemicetals B_{2a} and G_{2a}; (4) the cyclopentenone ring in aflatoxins B₁ and B₂ may be reduced to form the secondary alcohols, aflatoxicol and dihydroaflatoxicol.

Liver preparations of most species hydroxylate aflatoxins by pathway (1) to some extent (Portman, Plowman & Campbell, 1968; Schabert & Steyn,