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.

- Bearden, A. J. & Dunham, W. R. (1970). *Structure & Bonding*, 8, 1.
- Debrunner, P. G. (1970). In *Spectroscopic Approaches to Biomolecular Conformation*, p. 209. Ed. by Urry, D. W. Chicago: American Medical Association.
- Gibson, J. F., Hall, D. O., Thornley, J. H. M. & Whatley, F. R. (1966). *Proc. natn. Acad. Sci. U.S.A.* 56, 987.
- Johnson, C. E., Cammack, R., Rao, K. K. & Hall, D. O. (1971). *Biochem. biophys. Res. Commun.* 43, 564.
- Rao, K. K., Cammack, R., Hall, D. O. & Johnson, C. E. (1971). *Biochem. J.* 122, 257.

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

By M. GRIFFIN, J. P. KITCHER and P. W. TRUDGILL.
(Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth, Cards. SY23 3DD; U.K.)

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 900000, 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.

We wish to thank the Science Research Council for grants in support of this work.

- Dagley, S. & Johnson, P. A. (1963). *Biochim. biophys. Acta*, 78, 577.
- Hayaishi, O. (1969). *A. Rev. Biochem.* 38, 21.
- Hunt, A. L., Hughes, D. E. & Lowenstein, J. M. (1958). *Biochem. J.* 69, 170.
- Jones, A. H. & Trudgill, P. W. (1967). *Biochem. J.* 105, 31P.
- Kitcher, J. P. & Trudgill, P. W. (1970). *Biochem. J.* 118, 55P.
- Trudgill, P. W. (1969). *Biochem. J.* 113, 577.

Hydroxylation and Toxicity of Aflatoxins

By D. S. P. PATTERSON. (Central Veterinary Laboratory, Weybridge, Surrey, U.K.)

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,

1969; Patterson & Allcroft, 1970; Patterson & Roberts, 1970). Mouse, rabbit and rat livers demethylate aflatoxins (pathway 2) (Bassir & Emafo, 1970; Patterson & Roberts, 1971a), and aflatoxin hemiacetal is formed *in vitro* by guinea-pig, mouse and avian (chick, duck, quail and turkey) livers (pathway 3) (Patterson & Roberts, 1970, and unpublished work). Pathway (4), involving NADPH-dependent reduction, has recently been identified in avian and rabbit liver (Patterson & Roberts, 1971b). Transformations by pathways (1), (2) and (3) occur in crude or isolated microsomal preparations, but enzymes of pathway (4) are located in a soluble liver fraction (105 000g supernatant).

One-day-old ducklings are extremely susceptible to acute aflatoxin poisoning, and the toxin is probably taken up rapidly by the liver. However, there may be structural requirements for transport of aflatoxin-like molecules, because several closely related compounds are almost non-toxic compared with aflatoxin itself. Two such compounds are aflatoxin B_{2a} (Dutton & Heathcote, 1968) and aflatoxicol (Detroy & Hesseltine, 1968), and both are duckling liver metabolites. On the basis of results obtained *in vitro*, it may be calculated that the total metabolizing capacity of the liver is such that an LD₅₀ dose of aflatoxin B₁ would be completely transformed in under 50s. Consequently it is possible that, once formed intracellularly, primary toxic damage in the duckling is caused by one or both metabolites.

Rat liver has a smaller metabolic capacity, and it would take 64h to convert an LD₅₀ dose into aflatoxin M₁ and the demethylated toxin. Here the parent toxin must be regarded as the active molecule and so it appears that, depending on metabolic patterns, the toxic mechanism varies from species to species.

- Bassir, O. & Emafo, P. O. (1970). *Biochem. Pharmac.* **19**, 1681.
 Detroy, R. W. & Hesseltine, C. W. (1968). *Nature, Lond.*, **219**, 967.
 Dutton, M. F. & Heathcote, J. G. (1968). *Chem. Ind.* p. 418.
 Patterson, D. S. P. & Allcroft, R. (1970). *Fd Cosmet. Tox.* **8**, 43.
 Patterson, D. S. P. & Roberts, B. A. (1970). *Fd Cosmet. Tox.* **8**, 527.
 Patterson, D. S. P. & Roberts, B. A. (1971a). *Biochem. Pharmac.* (in the Press).
 Patterson, D. S. P. & Roberts, B. A. (1971b). *Fd Cosmet. Tox.* (in the Press).
 Portman, R. S., Plowman, K. M. & Campbell, T. C. (1968). *Biochem. biophys. Res. Commun.* **33**, 711.
 Schabert, J. C. & Steyn, M. (1969). *Biochem. Pharmac.* **19**, 2241.

Purification of Nicotinamide-Adenine Dinucleotide-Dependent Dehydrogenases by Affinity Chromatography

By KLAUS MOSBACH, HUGH GUILFORD, PER-OLOF LARSSON, ROLF OHLSSON and MARGARETHA SCOTT (introduced by J. E. SCOTT). (*Biochemical Division, Chemical Centre, University of Lund, P.O. Box 740, S-220 07, Lund, Sweden*)

We report the chemical binding of (a) NAD⁺ and (b) an AMP analogue to separate gel matrices, and the application of these functionalized gels to the separation of dehydrogenases by affinity chromatography (Mosbach, 1971).

For preparation (a) ϵ -aminohexanoic acid has been coupled to Sepharose 4B by the cyanogen bromide method (Axén, Porath & Ernback, 1967) and subsequently dicyclohexylcarbodi-imide was used to attach NAD⁺, probably via ester linkages (Larsson & Mosbach, 1971). For preparation (b) N⁶-(6-aminohexyl)-AMP (H. Guilford, P.-O. Larsson & K. Mosbach, unpublished work) has been linked directly to activated Sepharose 4B. In both cases a hexacarbon 'spacer' of about 7 Å length increased the steric availability of the cofactor and inhibitor moieties to the dehydrogenases studied, namely D-glyceraldehyde 3-phosphate-NAD oxidoreductase (EC 1.2.1.12) and L-lactate-NAD oxidoreductase (EC 1.1.1.27). Both enzymes were retained by columns packed with gel matrix, but could be eluted by solutions of NAD⁺.

It seems probable that the affinity of the bound AMP analogue for the dehydrogenases parallels the known action of free AMP as a competitive inhibitor (McPherson, 1970, and references cited therein). Further, the coenzyme NAD⁺ retains its binding capacity for NAD⁺-dependent dehydrogenases even when bound.

A 0.1 M-phosphate buffer solution, pH 7.0 (200 μ l), containing D-glyceraldehyde 3-phosphate-NAD oxidoreductase (0.4 mg, 45 U) and bovine serum albumin (1 mg) was applied to a column (11 cm $\times$ 1 cm) packed with preparation (a) containing nucleotide (17.5 μ mol). Albumin appeared in the early fractions whereas the enzyme was eluted only after the application of 10 mM-NAD⁺ (50 μ l). Of the total activity about 90% was recovered in a sharp peak. Similarly, with the same protein solution but preparation (b) containing nucleotide (37 μ mol) as column material and 0.15 mM-NAD⁺ as eluent the same elution pattern was observed. In blanks run with gels lacking nucleotides the dehydrogenases and albumin were eluted together.

Mixtures of D-glyceraldehyde 3-phosphate-NAD oxidoreductase and L-lactate-NAD oxidoreductase could also be resolved. Thus, when both were applied to a column containing preparation (b), elution with 0.15 mM-NAD⁺ afforded only the