With this method it was shown that the covalent binding of trypsin to CNBr-activated Sephadex stabilized the protein against the denaturing effect of concentrated urea solutions, in contradistinction to the similar agarose-bound trypsin, which behaved as the free trypsin. This agrees with activity measurements (Gabel *et al.*, 1970), the latter derivative being inactive in 8M-urea, as is the free enzyme, whereas Sephadex-bound trypsin retains most of its activity even after long storage in urea solutions.

The stabilization of the active surface of trypsin by Ca^{2+} , found for the free enzyme (Sipos & Merkel, 1970), was not observed with the Sephadex-bound enzyme. The protein in solution unfolds at 60°C in the presence of Ca^{2+} , as reflected by a red shift of the tryptophan fluorescence, whereas the fluorescence properties of Sephadex-bound trypsin did not change up to 70°C. At that temperature the fluorescence intensity increased, but no red shift was observable. This increase coincided with the loss of activity. It is suggested that the temperature-induced conforma-

tional transitions that inactivate trypsin in the absence and in the presence of Ca^{2+} , as well as the Ca^{2+} -dependent transition leading from native trypsin to a more compact structure (Sipos & Merkel, 1970), are not possible in Sephadex-bound trypsin owing to a more-or-less extensive cross-linking between the protein and the rigid carrier.

Attempts were made to identify those ϵ -amino groups that contribute to the stabilization of Sephadex-bound trypsin against denaturing influences.

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COMMUNICATIONS

Biosynthesis of Fatty Acids and Phenols by Stationary-Phase Cultures of *Aspergillus fumigatus*

By N. M. PACKTER and A. C. WARD (*Department of Biochemistry, University of Leeds, Leeds LS2 9LS, U.K.*)

Many fungi respond to deprivation of essential nutrients by enhancement of lipid synthesis (Borrow *et al.*, 1961) or secretion of acetate-derived phenols. *Aspergillus fumigatus* secretes 2,3,5-trihydroxytoluene under submerged cultural conditions. Synthesis of this metabolite does not involve a reductive step (but an oxygenase is required) and the complex concerned may possibly be a 'defective' fatty acid synthetase. However, Dimroth *et al.* (1970) have established that the synthetases responsible for forming 6-methylsalicylate and fatty acids are distinct in *Penicillium patulum*. Certain facets of fatty acid and phenol synthesis in *A. fumigatus* were therefore examined.

Homogeneous colonies of mycelium were transferred (before phenol secretion) to 5% (w/v) glucose solution. [$1\text{-}^{14}\text{C}$]Acetate (10 μCi) was added to each flask; three flasks (150 ml) were removed after a further 3 h, 7 h, 21 h and 28 h incubation. Lipids were isolated from the mycelium and saponified to generate the constituent fatty acids. They were purified and determined spectrophotometrically (Duncombe, 1963). Phenols were extracted as described by Packter

(1965); trihydroxytoluene was rechromatographed and crystallized. Fatty acid and ergosterol contents doubled during the test period. [^{14}C]Acetate was efficiently incorporated into these lipids (20 and 0.7%) within 3 h, but gave rise to insignificant labelling (0.05%) in the phenols from either medium or mycelium; carrier material was added to the early groups.

Next, [$1\text{-}^{14}\text{C}$]acetate was added to mycelium at 0.5, 5, 17, 28 and 48 h after transfer; each group was harvested 1 h later. Incorporation into fatty acids was 16.8, 32.5, 16.9, 20.1 and 18.2% for the individual groups; values for ergosterol were 0.7, 1.6, 0.4, 1.3 and 1.2%. Again, lipid and ergosterol content increased. Incorporation into phenols was low for groups 1 and 2 (0.3 and 0.7%), but considerable conversion occurred in the later groups (14.3, 13.6 and 13.1%).

The ability to form fatty acids at a late stage in the growth period on normal medium was verified by assay of the synthetase (Lynen, 1969). Its activity fell after exhaustion of nitrogen, but only to a value comparable with that of many other enzymes.

These results indicate that initiation of phenol synthesis is not a simple response to lack of nutrients. Presumably metabolic events that ensue later are responsible for the expression of 'aromatic synthetase' activity. However, fatty acid synthetase remains active.

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The Identity of Shikimate Dehydrogenase and Quinate Dehydrogenase in *Aspergillus niger*

By R. B. CAIN* (*Department of Medical Chemistry, Faculty of Medicine, University of Kyoto, Kyoto, Japan*)

Aspergillus niger, as well as members of several other fungal genera (Cain *et al.*, 1968; Ahmed & Giles, 1969), grows rapidly with shikimate or quinate as the sole carbon and energy source, and degrades these substrates through protocatechuate.

Growth of *Asp. niger* on either quinate or shikimate induced an NAD-mediated dehydrogenase active against both of these substrates. This enzyme was distinct from the constitutive NADP-linked dehydroshikimate reductase (EC 1.1.1.25), from which it was separated during purification. Although the enzyme exhibited different pH optima for shikimate and quinate and the kinetics of thermal denaturation at 47°C were detectably different when the extract was tested against these substrates, the dehydrogenase activity was probably catalysed by the same enzyme because: (a) at saturating concentrations addition of either shikimate or quinate to the assay did not increase the reaction rate when the other substrate was present alone; (b) there was no separation of the activities on a sucrose density gradient or by Sephadex G-200 chromatography; (c) both activities were strongly repressed when glucose was present in the medium and the extents of repression were identical for both activities; (d) the presence of inhibitors such as chloromercuribenzenesulphonate led to equal inhibition of each activity; (e) the ratio of the dehydrogenase activities with shikimate and quinate remained identical when the enzyme was purified 100-fold through a four-stage scheme to homogeneity by polyacrylamide-gel electrophoresis.

Among several u.v.-induced mutants of this strain of *Asp. niger*, inability to grow with shikimate was paralleled by loss of the ability to grow on quinate medium. One mutant lacking dehydrogenase activity was inactive when extracts were tested both with shikimate and with quinate. Revertants from this mutant simultaneously regained both shikimate

dehydrogenase and quinate dehydrogenase activities.

The dehydrogenase had K_m for NAD⁺ 0.42 and 0.26 mM when shikimate and quinate respectively were saturating and K_m for shikimate and for quinate 11.5 and 6.25 mM respectively in the presence of excess of NAD⁺. The molecular weight was 23 000 ± 500 (mean ± S.E.M. of six determinations) by Sephadex G-100 chromatography. Only growth substrates containing a shikimate or a quinate moiety (e.g. chlorogenate) induced the dehydrogenase.

This reliance in *Asp. niger* of shikimate dehydrogenase and quinate dehydrogenase activities on a single enzyme closely resembles the situation in *Acinetobacter calco-aceticus* (Tresguerres *et al.*, 1970), but the regulation of the catabolic pathway is quite distinct.

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Metabolism of Shikimate and Quinate by *Aspergillus niger* and its Regulation

By R. B. CAIN* (*Department of Medical Chemistry, Faculty of Medicine, University of Kyoto, Kyoto, Japan*)

The metabolism of the widely occurring plant acids shikimate and quinate by *Aspergillus niger* is initiated by a dehydrogenase acting on either substrate and forming the corresponding 5-dehydroshikimate and 5-dehydroquininate respectively (Cain, 1972). These two products are interconverted by the enzyme dehydroquinase (EC 4.2.1.10), although the equilibrium constant strongly favours 5-dehydroshikimate formation, and 5-dehydroshikimate is converted into protocatechuate by an inducible dehydrase (Gross, 1958).

Giles *et al.* (1967) and Ahmed & Giles (1969) have shown that *Neurospora crassa* and several other fungi synthesized two dehydroquinases, one of which was associated with the enzyme aggregate in the common aromatic-amino acid-biosynthetic pathway and the other appeared only when quinate or a precursor was the principal carbon source.

Examination of glucose-grown mycelial extracts of *Asp. niger* by sucrose-density-gradient centrifugation revealed the biosynthetic dehydroquinase ($s_{20,w}$ 14.2S) with K_m for 5-dehydroquininate 46 μM.

* Present address: Biological Laboratories, University of Kent, Canterbury, Kent, U.K.

* Present address: Biological Laboratories, University of Kent, Canterbury, Kent, U.K.