The Regulation of the Enzymes of Aromatic-Ring Fission in an Actinomycete

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In addition to our fungal investigations we have also examined certain actinomycetes for the regulatory control of aromatic metabolism and given particular attention to *Nocardia opaca*, an organism whose nutritional spectrum approaches that of the pseudomonads. Growth of *N. opaca* on either *p*-hydroxybenzoate or benzoate elicited the induction of all the enzymes of both protocatechuate and catechol pathways, although these pathways are biochemically quite distinct. Similarly, growth at the expense of *cis-cis*-muconate (catechol is extremely toxic to this bacterium) by a permeability mutant of *N. opaca* also induced the complete enzymic complement of both pathways. Protocatechuate itself, however, elicited only the protocatechuate pathway.

The induction pattern for the protocatechuate pathway showed that protocatechuate oxygenase, β -carboxymuconate-lactonizing enzyme, γ -carboxymuconate decarboxylase, enol lactone hydrolase and β -oxoadipate transferase were all co-ordinately induced. Similarly there was a co-ordinate pattern of induction between muconolactone isomerase, enol lactone hydrolase and the transferase in the benzoate pathway. Although catechol oxygenase was product-induced by *cis-cis*-muconate, both it and muconate-lactonizing enzymes were apparently independently regulated.

N. opaca formed two distinct but isofunctional enol lactone hydrolases, one characteristic of the catechol pathway, the other of the protocatechuate pathway. They differed in the kinetics of their thermal denaturation and in their pattern of elution from Sephadex G-200 columns, the benzoate-induced enzyme being more heat-sensitive and of lower molecular weight. In contrast, benzoate and *p*-hydroxybenzoate induced an identical β -oxoadipate transferase. The transferases induced by either substrate had identical thermal denaturation characteristics, identical molecular weight, the same K_m for β -oxoadipate and were inhibited to the same extent by adipate (K_i 15 mM).

Mutants permeable to *cis-cis*-muconate also grew exponentially on β -oxoadipate and contained all the enzymes operative in the protocatechuate pathway, but only muconolactone isomerase of the catechol pathway. This evidence suggests that β -oxoadipate (or its CoA derivative) was the inducer of the protocatechuate block of enzymes together with muconolactone isomerase and the (distinct) enol lactone hydrolase of the benzoate pathway. Further weight was lent to this hypothesis

by the results obtained by growth on adipate, which, although metabolically without function in aromatic compound catabolism, induced all the enzymes of the protocatechuate pathway although only to some 10% of the activities obtained with *p*-hydroxybenzoate. The transferase induced by adipate is distinct from that elicited by benzoate, *p*-hydroxybenzoate or β -oxoadipate.

This work was supported by the Agricultural Research Council. D.L.R. was in receipt of a Science Research Council post-graduate studentship.

A Dietary Factor Required for the Induction of Hepatic Microsomal Cytochrome P-450 by Phenobarbitone

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Cytochrome P-450 is a central component of the detoxication enzymes of hepatic microsomes, which catalyse the hydroxylation of a wide variety of compounds. These include endogenous substrates, such as steroids, but also many of the foreign compounds that constitute the chemical environment. Among these are drugs (e.g. barbiturates), pollutants (e.g. benzopyrene) and dietary components, both natural (e.g. steroids and terpenoids) and man-made (e.g. DDT).

Many of the substrates of the enzyme are inducers of it, and we have investigated the factors that control the concentration of cytochrome P-450 in the rat and determine its response to inducers.

Administration of phenobarbitone as a 0.1% solution in the drinking water (Marshall & McLean, 1969) for 7 days or longer raises the concentration of cytochrome P-450 from 20 to 120 nmol/g. of liver in animals fed on a stock diet (41B). In animals fed on a purified diet containing 15% of casein (McLean & McLean, 1966) the concentration of cytochrome P-450 is 14 nmol/g. of liver and is increased to only 60 nmol/g. by phenobarbitone.

Since the 15%-casein diet is nutritionally adequate, allowing growth at the rate of 5 g./day or more, this result suggests that purified diets are deficient in a factor present in diet 41B. This factor is not itself a powerful inducer but allows a greater induction by phenobarbitone, i.e. the combined effect of the permissive factor and phenobarbitone is multiplicative rather than additive. We have found this factor to be present in diet 41B, ethereal extracts of diet 41B and whole liver.

We have previously described a separate permissive effect dependent on dietary protein content (Marshall & McLean, 1969). In animals fed on a

protein-deficient purified diet (3% casein), phenobarbitone increases cytochrome *P*-450 only from 6 to 30nmol/g. of liver. This effect is only due to dietary protein, however, and re-feeding experiments indicate that the control of induction in protein-deficient animals may be altered so as to divert available protein away from less essential functions, of which the synthesis of cytochrome *P*-450 seems to be one.

The permissive effect of the factor in diet 41B has not been described before; its nature and possible mode of action will be discussed.

This work was supported by a grant from the Medical Research Council.

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Gut Bacteria and the Metabolism of Cyclamate in the Rat

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It has been established that cyclohexylamine is a minor metabolite of cyclamate (cyclohexylsulphamate) in the urine in man and animals (see, e.g., Golberg, Parekh, Patti & Soike, 1969; Oser, Carson, Nogin & Sonder, 1968).

We have found that Wistar albino rats kept on a normal diet and water excreted very little cyclohexylamine after a single oral dose of cyclamate, but rats kept for 3 months on the same diet but with water containing 0.5% calcium cyclamate (about 550mg./kg./day) developed the ability to convert cyclamate into cyclohexylamine. The latter rats (22) were given a single oral dose of calcium cyclamate (200mg.) and the urinary output of cyclohexylamine was determined by g.l.c. The urinary excretion of cyclohexylamine in 24hr. ranged from 0.7 to 35% of the dose (seven rats excreted more than 10% and 15 less than 10%). The cyclamate-treated rats (three) were given a single oral dose of [¹⁴C]cyclamate (50mg./kg.) and they excreted 93 (88–97)% of the ¹⁴C in 3 days, 64% in the urine and 27% in the faeces. In the first 24hr. after dosing an average of 58% of the ¹⁴C was excreted in the urine, 33% as unchanged cyclamate, 22% as cyclohexylamine and 3% as probably conjugated cyclohexylhydroxylamine. The faeces contained cyclamate and cyclohexylamine.

Three cyclamate-pretreated rats were given simultaneously [¹⁴C]cyclamate (10mg./kg.) intra-

peritoneally and unlabelled cyclamate (320mg./kg.) orally. The 24hr. urine was examined for cyclohexylamine by g.l.c. and scintillation counting. The conversion of [¹⁴C]cyclamate (given intraperitoneally) into cyclohexylamine was 0.9%, whereas that of unlabelled cyclamate (given orally) was 38%. About 90% of injected cyclamate was excreted in the urine in 24hr. compared with 58% when given orally.

When [¹⁴C]cyclamate was incubated for 1.5hr. with preparations of liver, kidney, spleen or blood from cyclamate-pretreated rats the formation of cyclohexylamine was no different from that of controls, i.e. less than 0.05%. The contents of the hindgut of the rats, however, converted 0.8–3.5% into cyclohexylamine under anaerobic conditions and 0.4–2.5% under aerobic conditions.

When the pretreated rats were taken off the cyclamate-containing diet, the ability to produce cyclohexylamine from cyclamate fell to a low level in 5 days.

These experiments suggest that the conversion of cyclamate into cyclohexylamine in the rat is carried out by the gut flora, which acquire this ability when the diet contains cyclamate. This ability is largely lost on removing cyclamate from the diet.

The work was supported by the International Sugar Research Foundation Inc.

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The Physical Properties and Mechanism of Action of Deoxyxoglucarate Dehydratase from *Pseudomonas acidovorans*

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Deoxyxoglucarate dehydratase catalyses the conversion of D-4-deoxy-5-oxoglucarate into 2,5-dioxovalerate (2-oxoglutarate semialdehyde), CO₂ and water (Jeffcoat, Hassall & Dagley, 1968).

We report here the following physical properties of the enzyme from *Pseudomonas acidovorans* N.C.I.B. 10013 grown on glucarate: *S*_{20,w}⁰, 7.0s; *D*_{20,w}, 5.82 × 10⁻⁷ cm.²/sec.; $\bar{v}$, from the amino acid composition, 0.74 cm.³/g.; *E*_{cm}^{1%} at 280nm., 8.3; mol.wt. by sedimentation equilibrium, 118000; *S*_{20,w}⁰ in 6M-urea, 2.3s; *S*_{20,w}⁰ in 6M-guanidine