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.

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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