Degradation of *p*-Methoxybenzoate by Cell-Free Extracts of *Pseudomonas fluorescens*

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Bacteria grown on *p*-methoxybenzoate are induced to metabolize *p*-methoxybenzoate, *p*-hydroxybenzoate and protocatechuate (Cartwright & Buswell, 1969; Cartwright *et al.*, 1970).

A cell-free system from *Pseudomonas fluorescens* strain PM3, grown on *p*-methoxybenzoate as sole carbon and energy source, catalysed the oxidative demethylation, hydroxylation and subsequent ring cleavage of *p*-methoxybenzoate. Demethylation, to yield *p*-hydroxybenzoate, involved absorption of 1 mol of O₂/mol of *p*-methoxybenzoate and required reduced nicotinamide nucleotide (either NADH or NADPH) as cofactor. *p*-Hydroxybenzoate, the first detectable intermediate, was hydroxylated to protocatechuate by an NADPH-dependent *p*-hydroxybenzoate hydroxylase (Hosokawa & Stanier, 1966) with the absorption of 1 mol of O₂/mol of substrate. Protocatechuate was oxidized by *ortho* fission to 3-oxoadipate.

The methyl group of *p*-methoxybenzoate was removed as formaldehyde, and oxidized to formate and carbon dioxide by formaldehyde dehydrogenase, which required GSH and NAD⁺, and formate dehydrogenase, which required NAD⁺. Each step accounted for the absorption of 0.5 mol of O₂/mol of substrate. All enzymic activity was present in the supernatant fraction prepared by centrifugation of sonically disrupted cells at 270000g for 3 h.

The ether-cleaving enzyme in *Ps. fluorescens* strain PM3 is specific for a substituent *para* to a carboxyl group. Of the methoxylated compounds examined only *p*-methoxybenzoate, isovanillate (3-hydroxy-4-methoxybenzoate) and veratrate (3,4-dimethoxybenzoate), resulting in the accumulation of vanillate, were oxidized. It is noteworthy that the demethylase system catalysed the oxidation of piperonylate (3,4-methylenedioxybenzoate) with the absorption of 2 mol of O₂/mol of substrate. Activity was NADH-dependent and resulted in the accumulation of Rothera-positive material, the 2,4-dinitrophenylhydrazone of which was chromatographically identical with the laevulinate derivative.

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Studies on the Biosynthesis of Cyclic Depsipeptides in *Serratia marcescens*

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Almost all the important features of the biosynthesis of cyclic depsipeptides are unknown (Taylor, 1970); these include the activation of the component moieties, the mechanism of formation and the way in which the order of the residues is controlled. The biosynthesis of these compounds is of particular interest since studies with chloramphenicol (Katz *et al.*, 1965) have shown that cyclic depsipeptides are formed by a system that is quite different to that of protein synthesis and probably non-ribosomal. The cyclic depsipeptides of *Serratia marcescens* (Bermingham *et al.*, 1970a) are among the simplest representatives of this group of lipids, and the major component, serratamolide (which contains only L-serine and D-3-hydroxydecanoate), offers one of the best opportunities for biosynthetic studies. These have been attempted by (a) feeding of radioactive precursors to *S. marcescens* and (b) inhibition studies with streptomycin (which blocks serratamolide biosynthesis; Bermingham *et al.*, 1970b).

3-Hydroxy[¹⁴C]decanoate (isolated from the rhamnolipid of [¹⁴C]acetate-grown *Pseudomonas aeruginosa*; Hauser & Karnovsky, 1954) and [¹⁴C]acetate were fed to *S. marcescens* N.T.C.C. 1377 in the presence and in the absence of streptomycin (10 µg/ml of medium) and the resulting radioactive lipids were analysed by t.l.c. as previously described (Bermingham *et al.*, 1970b). Radioactivity was incorporated readily from acetate into serratamolide and this incorporation was blocked by streptomycin, but no incorporation into cyclic depsipeptide was found from 3-hydroxy[¹⁴C]decanoate even though this compound was taken up and metabolized into phosphatidylethanolamine and phosphatidylglycerol. Subsequent feeding of [1-¹⁴C]octanoate did give rise to [¹⁴C]serratamolide. It appears that some precursor of 3-hydroxydecanoate rather than the acid itself is utilized for serratamolide biosynthesis.

G.l.c. analysis of the fatty acids from a purified cyclic depsipeptide fraction of *S. marcescens* grown in the presence of streptomycin showed complete inhibition of the synthesis of 3-hydroxydecanoate with the simultaneous appearance of two non-hydroxy fatty acids (not present in the control cells), which were identified as dodecanoate (laurate) and tetradecanoate (myristate). It may be that, in the absence of a demand for 3-hydroxydecanoate for serratamolide production, this acid is not released from the fatty acid-synthesizing complex.

We have previously shown that L-[¹⁴C]serine

may be incorporated directly into serratamolide (Birmingham *et al.*, 1970b). Whether the linear amide, serratamic acid (3-hydroxydecanoylserine), is an intermediate has yet to be determined, but preliminary results from feeding of [^{14}C]serratamic acid seem to indicate a breakdown of this compound to [^{14}C]serine before reincorporation into serratamolide rather than a direct synthesis by twinning of serratamic acid.

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Metabolism of Linear Alkylbenzenesulphonates by a *Vibrio* sp.

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Linear alkylbenzenesulphonates presently constitute a major group of detergent compounds in domestic and industrial usage. Although the biodegradability of these compounds has been known for many years, research has generally been limited to intact-cell studies with mixed microflora populations derived from sewage or natural river water (Swisher, 1970). Pure-culture studies (Willets & Cain, 1970; Cain *et al.*, 1972) have shown that certain bacteria and fungi can completely degrade the linear alkylbenzenesulphonate molecule, but the *Vibrio* sp. described here, although growing with dodecylbenzene-*p*-sulphonate as sole carbon and energy source, utilized only the alkyl side chain. This inference was deduced from the following results: (a) there was a rapid and immediate loss of the side chain, as indicated by the disappearance of Methylene Blue-positive substances (Longwell & Maniece, 1955); (b) there was no loss of the u.v. absorption at 225 nm due to the aromatic ring of dodecylbenzene-*p*-sulphonate, or of the sulphonate group as sulphite; (c) continuous sampling of cultures growing at the expense of dodecylbenzene-*p*-sulphonate (30 p.p.m.) followed by micro-desulphonation and g.l.c. of the desulphonation products showed that over 5 days

the loss of substrate was accompanied by the accumulation of large quantities of sulphophenylacetate and sulphophenylbutyrate together with small amounts of the hexanoate and octanoate homologues. In the same way a linear alkylbenzenesulphonate with an odd-numbered side chain (undecylbenzene-*p*-sulphonate) was degraded to odd-numbered sulphophenylalkanoates.

These results suggest that metabolism of linear alkylbenzenesulphonates by the *Vibrio* sp. involved ω - and β -oxidation of the side chain (cf. Huddleston & Allred, 1963). Cultures of the *Vibrio* sp. degraded undecylbenzene-*p*-sulphonate and its ω -oxidation product, sulphophenylundecanoate, at almost identical rates, giving similar growth yields (approx. 2×10^6 cells/ml) and accumulating identical degradation products on g.l.c. Further, the difference in O_2 uptake of approx. 1.5 mol of O_2 /mol between undecylbenzene-*p*-sulphonate and sulphophenylundecanoate when oxidized by adapted washed-cell suspensions was consistent with the net requirement for ω -oxidation of an alkane hydrocarbon to the corresponding alkanoate.

Enzymic characteristics associated with growth on linear alkylbenzenesulphonates as distinct from succinate included enhanced activities of acyl-CoA synthetase (EC 6.2.1.3) active against a wide range of alkanoates, phenylalkanoates and sulphophenylalkanoates and a marked elevation of the amounts of isocitrate lyase (EC 4.1.3.1) and malate synthase (EC 4.1.3.2), consistent with the organism producing C_2 units by β -oxidation of the alkyl chain.

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Induction of *petite* Mutants in Yeast by Starvation in Glycerol

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A number of physical and chemical treatments can cause practically complete conversion of the yeast *Saccharomyces cerevisiae* into respiratory-

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