Aminoacetone was formed from L-threonine in cell-free extracts of *B. subtilis* grown on nutrient broth. The amino ketone was then alternatively reduced to 1-aminopropan-2-ol or transaminated to methylglyoxal. Whereas 1-aminopropan-2-ol was apparently not further metabolized, methylglyoxal was oxidized to pyruvate, either directly or indirectly via D-lactate or lactaldehyde.

Enzymes of the 'amino ketone pathway' of threonine metabolism that were studied include L-threonine dehydrogenase, aminoacetone aminotransferase (Higgins, Turner & Willetts, 1967), aminoacetone reductase, 1-aminopropan-2-ol dehydrogenase (Turner, 1966), methylglyoxal oxidase (Monder, 1965; Higgins & Turner, 1969), methylglyoxalase (Racker, 1952; Higgins & Turner, 1969), methylglyoxal reductase (Ting, Miller & Sellinger, 1965), D-lactate dehydrogenase (Brin & Dunlop, 1965) and lactaldehyde oxidase. Additional enzymes studied were L-threonine dehydratase, L-threonine aldolase, 2-amino-3-oxobutyrates-CoA ligase (McGilvray & Morris, 1969), the 'glycine-cleavage system' (Sagers & Gunsalus, 1961), L-serine hydroxymethyltransferase and L-serine dehydratase. The properties of these enzymes partially purified from *B. subtilis* are consistent with the operation of the amino ketone pathway. Radiotracer experiments with L-[U-¹⁴C]threonine support this conclusion, as also do growth and enzyme-induction results.

The effect of growth conditions on enzyme activities was examined with simple synthetic media. The aminoacetone-dependent pathway predominated in *B. subtilis* grown on such media containing relatively low concentrations of L-threonine. Whereas L-threonine dehydrogenase, methylglyoxalase and methylglyoxal oxidase were constitutive, at high activities, the formation of all other enzymes was markedly induced by the amino acid. The additional development of a threonine aldolase-dependent pathway was induced by higher threonine concentrations. Threonine aldolase and the enzymes of glycine metabolism via serine were all markedly affected. Catabolic pathways involving threonine dehydratase or 2-amino-3-oxobutyrates-CoA ligase did not appear to operate in *B. subtilis*, even when L-threonine served as the major source of carbon for growth.

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2,3-Dihydroxybenzoate 3,4-Oxygenase from *Pseudomonas fluorescens*: Determination of the Site of Ring Cleavage with a Substrate Analogue

By D. W. RIBBONS and P. J. SENIOR.* (*Department of Biochemistry, University of Miami Medical School, and Howard Hughes Medical Institute, Miami, Fla. 33152, U.S.A.*)

The oxidation of 2,3-dihydroxybenzoate has been shown to proceed in fungi by decarboxylation to catechol and an intradiol oxygenative cleavage of this product to yield eventually β -oxoadipate (Terui, Enatsu & Tabata, 1961; Subba Rao, Moore & Towers, 1967). It has been shown that *Pseudomonas fluorescens* 23D-1 is able to cleave the benzenoid ring of 2,3-dihydroxybenzoate by an extradiol cleavage to yield α -hydroxymuconic semialdehyde and carbon dioxide (Ribbons, 1966a). Catechol was excluded as an intermediate in this transformation, even though α -hydroxymuconic semialdehyde is also the product of the extradiol ring cleavage of catechol by catechol 2,3-oxygenase (Dagley, Evans & Ribbons, 1960; Kojima, Itada & Hayaishi, 1961). There was, however, doubt about the site of the extradiol cleavage of 2,3-dihydroxybenzoate, since α -hydroxymuconic semialdehyde could arise by cleavage of C₍₁₎-C₍₂₎ or C₍₃₎-C₍₄₎ bonds (Ribbons & Watkinson, 1968).

An analogue of 2,3-dihydroxybenzoate, 2,3-dihydroxy-*p*-toluate, has been isolated as co-oxidation product from *p*-xylene fermentations (Raymond, Jamison & Hudson, 1967) and a sample of this compound (kindly provided by R. Raymond) was tested as a substrate for 2,3-dihydroxybenzoate oxygenase. 2,3-Dihydroxy-*p*-toluate is oxidized by 2,3-dihydroxybenzoate-induced cells and extracts of *Ps. fluorescens* with the consumption of 1 mol of oxygen and release of 1 mol of carbon dioxide and the stoichiometric formation of 2,6-dioxoheptenoic acid; this acid is not further metabolized by *Ps. fluorescens*. The isolated product was characterized by

* Present address: Department of Biochemistry, University of Hull, Kingston-upon-Hull, U.K.

u.v., visible and i.r. spectra, by its conversion in acidic ammonium acetate into 6-methyl-2-picolinate, and by its rapid hydrolysis by extracts of *Ps. aeruginosa* T1 to yield pyruvate and acetate (Ribbons, 1966b). 2,6-Dioxoheptenoate is also known to be the product of 3-methylcatechol oxidation catalysed by catechol 2,3-oxygenase of other pseudomonads (Dagley, Chapman, Gibson & Wood, 1964; Ribbons, 1966a, b; Catelani, Fieocchi & Galli, 1968). 3-Methylcatechol is not oxidized by cells or extracts of 2,3-dihydroxybenzoate-grown *Ps. fluorescens* 23D-1, nor is it formed anaerobically from 2,3-dihydroxy-*p*-toluate. 2,3-Dihydroxy-*p*-toluate is not an inducer for the oxygenase, nor a growth substrate for *Ps. fluorescens*. The results are consistent only with a ring cleavage of 2,3-dihydroxy-*p*-toluate in the 3,4-position, and thereby indicates that the site of cleavage of 2,3-dihydroxybenzoate is at the 3,4-position.

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Inhibition by Streptomycin of the Biosynthesis of Cyclic Depsipeptides in *Serratia marcescens*

By MARGARET A. C. BERMINGHAM, B. S. DEOL and J. L. STILL. (Department of Biochemistry, University of Sydney, N.S.W. 2006, Australia)

An acetone-soluble lipoamino acid, serratamic acid, was isolated from *Serratia marcescens* during a study of the biosynthesis of the tripyrrolic pigment prodigiosin (Cartwright, 1955); it was shown to consist of a linear amide of L-serine and β -hydroxydecanoic acid (Cartwright, 1957). A dimer of serratamic acid, serratamolide, was later discovered (Wasserman, Keggi & McKeon, 1961) and proved to have a cyclic depsipeptide structure that gave rise to two molecules of serratamic acid on mild alkaline hydrolysis. It has been speculated that serratamolide is the naturally occurring compound, serratamic acid being an artifact of extraction procedures.

Recent experiments in this laboratory have confirmed this speculation and strongly suggest that two further cyclic depsipeptides (consisting of serine esterified to β -hydroxydecanoic acid and β -hydroxydodecanoic acid respectively) are also present (M. A. C. Bermingham, B. S. Deol & J. L. Still, unpublished work).

No connexion has been established thus far between the biosynthesis of these compounds and prodigiosin formation, but 'lipoamino acid' content of *S. marcescens* is greatest in highly pigmented strains, and growth conditions (e.g. temperature, carbon source and pH) that favour pigment production also favour lipoamino acid biosynthesis (Deol, Bermingham & Still, 1969).

The formation of prodigiosin in the mutant strain 933 is 75% inhibited by streptomycin (10 μ g/ml of medium) although growth is not affected (Williams & Gott, 1964). We have investigated the effect of streptomycin on the biosynthesis of prodigiosin and cyclic depsipeptide (red strain 330, bacto-peptone medium) and find that very low concentrations (2.5–5.0 μ g/ml of medium) do not affect growth or pigmentation under these conditions but cause a sevenfold decrease in cyclic depsipeptide content (measured as acetone-soluble bound serine). In the white mutant strain 214 (no pigmentation) biosynthesis of cyclic depsipeptide is completely inhibited by streptomycin.

Extraction and fractionation of lipid from control (C) and streptomycin-grown (S) cells indicate that, although total lipid weight [chloroform-methanol (2:1, v/v)-soluble] is unaffected (C, 138.8mg/g dry wt. of cells; S, 131.6mg/g dry wt. of cells), the neutral lipid (acetone-soluble, containing cyclic depsipeptides) is decreased (C, 62.2mg/g; S, 37.8mg/g) and consequently the polar lipid is increased. The phosphorus content of the total lipid is increased from 1.58% (C) to 3.76% (S).

T.l.c. of the acetone extracts [silica gel G; chloroform-methanol-7M-NH₃ (65:25:4, by vol.)] confirmed that the cyclic depsipeptide content was markedly decreased by streptomycin. When feeding experiments were carried out with [U-¹⁴C]serine (specific radioactivity 667 μ Ci/mg) at a concentration of 0.012 μ Ci/ml of medium, radioactivity was incorporated very rapidly into cyclic depsipeptides, and this incorporation was decreased almost to zero by streptomycin treatment. The results will be discussed.

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