A New Pathway for the Biosynthesis of Spermidine

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The only pathway known for the biosynthesis of spermidine is from putrescine and S-adenosylmethionine. S-Adenosylmethionine is decarboxylated, and the aminopropyl group of the product is transferred to putrescine to form spermidine. The enzymes of this pathway, S-adenosylmethionine decarboxylase (EC 4.1.1.50) and aminopropyltransferase (EC 2.5.1.16), have been found in a number of bacteria, yeasts and mammalian cells (Tabor & Tabor, 1972; Pösö et al., 1976). In this report it is shown that Micrococcus denitrificans and Rhodopseudomonas spheroides do not form spermidine in this way. They form it from putrescine and L-aspartic β-semialdehyde.

M. denitrificans synthesizes and excretes three siderochromes, two of which contain spermidine, when grown in media deficient in iron (Tait, 1975). In preliminary experiments the siderochromes containing spermidine were labelled after adding to cultures L-[U-14C]arginine or DL-[5-14C]ornithine, known precursors of putrescine or [1,4-14C]putrescine itself, but not after adding L-[U-14C]methionine. S-Adenosylmethionine decarboxylase activity could not be detected in cell-free extracts. These results suggested that M. denitrificans might make spermidine by a novel route.

M. denitrificans was grown anaerobically in a defined nitrate medium deficient in iron (Tait, 1975), and R. spheroides was grown semi-anaerobically in the light as described by Neuberger et al. (1973). Cells were harvested by centrifugation, resuspended in 0.05M KH2PO4/NaOH buffer, pH 7.5, radioactive and unlabelled compounds were added, and suspensions were incubated at 30°C. Suspensions of R. spheroides were illuminated. Concentrated suspensions of cells were disrupted by ultrasonication and centrifuged at 105000g for 90 min. The supernatants were used as enzyme, either before or after dialysis. After incubation, cells and enzyme extracts were extracted with 10% (w/v) trichloroacetic acid. These extracts were saturated with Na2SO4, adjusted to pH 12–13 with NaOH, and the amines were extracted into butan-1-ol. The amines were separated by electrophoresis or chromatography on paper (Herbst et al., 1958). Quantitative determination of the amines was done as described by Russell et al. (1970), and their radioactivity was measured by liquid-scintillation spectrometry.

M. denitrificans and R. spheroides both contain about five times more spermidine than putrescine. Suspensions of these organisms formed radioactive spermidine from L-[U-14C]arginine, DL-[5-14C]ornithine and [1,4-14C]putrescine, but not when incubated with L-[U-14C]methionine and unlabelled putrescine. Enzyme extracts did not form radioactive spermidine from [1,4-14C]putrescine and S-adenosylmethionine. Of a number of labelled compounds incubated with cell suspensions in the presence of putrescine, L-[1-14C]aspartate and 14C-labelled fumarate gave the highest incorporations of radioactivity into spermidine; [2,3-14C]fumarate was twice as effective as [1,4-14C]-fumarate. It was concluded that a metabolic product of L-aspartate, possibly L-aspartic β-semialdehyde, might be the donor of the aminopropyl group of spermidine. In bacteria L-aspartic β-semialdehyde is an intermediate in the biosynthesis of L-homoserine (Fig. 1) and a number of other amino acids (cf. Greenberg, 1969). It is known (Gibson et al., 1962) that R. spheroides contains L-aspartate kinase (EC 2.7.2.4), an NADP+-dependent L-aspartic β-semialdehyde dehydrogenase (EC 1.2.1.11), and an NADP+-dependent L-homoserine dehydrogenase (EC 1.1.1.3), and that extracts can make L-aspartic β-semialdehyde from L-aspartate or from L-homoserine (Fig. 1).

Labelled spermidine was formed on incubating enzyme extracts from M. denitrificans and from R. spheroides with [1,4-14C]putrescine and L-aspartate (or putrescine and L-[U-14C]aspartate) plus ATP, Mg2+, NADPH and dithiothreitol, or with [1,4-14C]-
**Fig. 1. Pathway for the biosynthesis of spermidine in M. denitrificans and R. spheroides**

Putrescine and L-homoserine (or putrescine and L-[U-\(^{14}\)C]homoserine) plus NADP\(^+\). NADH and NAD\(^+\) could not replace NADPH and NADP\(^+\). Pyridoxal phosphate stimulated activity by a factor of about 2. In experiments with L-[U-\(^{14}\)C]aspartate and L-[U-\(^{14}\)C]homoserine the amounts of radioactivity incorporated into spermidine were three-quarters of those incorporated in experiments with [1,4-\(^{14}\)C]putrescine of the same specific radioactivity. \(^{14}\)CO\(_2\) was formed from L-[U-\(^{14}\)C]homoserine only in the presence of putrescine and NADP\(^+\).

L-Aspartic \(\beta\)-semialdehyde was synthesized by the method of Neuberger & Tait (1962). Labelled spermidine was formed on incubating enzyme extracts with [1,4-\(^{14}\)C]putrescine, NADPH and L-aspartic \(\beta\)-semialdehyde. NADH could not replace NADPH. Treatment of the enzyme extract from *M. denitrificans* with protamine sulphate and then with (NH\(_4\))\(_2\)SO\(_4\) gave a preparation that formed little or no spermidine from putrescine, L-aspartic \(\beta\)-semialdehyde and NADPH unless pyridoxal phosphate was added. Chromatography and electrophoresis of trichloroacetic acid extracts from incubations lacking only pyridoxal phosphate showed that a radioactive ninhydrin-positive compound was formed. Unlike spermidine, it was not extracted into butan-1-ol from alkaline solution. This compound is probably a basic amino acid, 'carboxyspermidine', formed by reduction of the condensation product of putrescine and L-aspartic \(\beta\)-semialdehyde.

The results reported here show that *M. denitrificans* and *R. spheroides* synthesize spermidine by a new pathway (Fig. 1). A Schiff base is probably formed between putrescine and L-aspartic \(\beta\)-semialdehyde; it is then reduced in an NADPH-dependent reaction to form 'carboxyspermidine', which is then decarboxylated by a pyridoxal phosphate-dependent enzyme to yield spermidine.
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Biphasic Interactions between a Neurospora crassa Glutamate Dehydrogenase and Reduced Nicotinamide–Adenine Dinucleotide Phosphate

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The NADP⁺-specific glutamate dehydrogenase (EC 1.4.1.4) from wild-type Neurospora crassa has been shown to exist in a predominantly active conformation in solutions above pH 7.1 and in an inactive conformation at more acid pH values. The presence of NADPH shifts the equilibrium towards the inactive conformation (West et al., 1967). This enzyme has been shown by fluorimetric studies (at equilibrium) to form a stable binary complex with NADPH (Gore et al., 1972). Further studies (Gore & Iwatsubo, 1975) have suggested that the system is unstable and that the original binary complex formed, for example, at pH 7.5 undergoes a structural transition before attaining an equilibrium state.

The studies by Gore & Iwatsubo (1975) have shown that a structural transition of reasonable magnitude (80 kJ/mol) will take place after the formation of an initial binary complex when a solution of enzyme in the active conformation is rapidly mixed with NADPH at pH values between 6.5 and 8.5. The signal change from this presumed conformational change had a maximum amplitude when enzyme at pH 8.0 or above was mixed 1:6 (mol/mol) with a solution of NADPH at pH 6.5. Since these are the conditions that would cause a maximum loss in catalytic activity of the enzyme, it is suggested either that as the enzyme assumed the inactive conformation it obtained a greater affinity for NADPH or that a change in the configuration of the enzyme-bound NADPH took place altering its spectral properties. The cause of this signal change has been investigated to try and identify the nature of the control mechanism exerted by NADPH.

By use of a solution of enzyme preincubated in 10 mm-Tris/HCl buffer, pH 8.1, and mixing this with a sixfold excess of NADPH in 50 mm-potassium phosphate buffer, pH 6.5, it was possible to observe the binding of NADPH to the active conformation at pH 6.5 where the inactive conformation normally prevails. Alternatively, it was possible to treat the inactive conformation (enzyme preincubated at pH 6.5) with an excess of NADPH in Tris/HCl buffer, pH 8.5 (final pH 8.1), thus momentarily trapping the inactive conformation in an environment normally associated with the catalytically active species of the enzyme. A rapid formation of a binary complex took place in both situations, followed by a slower relaxation of enzyme conformation to that which would normally predominate in the new environment. A typical situation is demonstrated in Fig. 1. Here a 14 µm solution of active enzyme in 10 mm-Tris/HCl buffer, pH 8.1, was mixed with a solution of NADPH (30 µm) to give a final pH of 6.5. The rapid initial binding was analysed as a first-order process k⁺ (s⁻¹) when the final NADPH concentration was at least 10 times that of the enzyme. The rate, k⁺ (s⁻¹), was then plotted against the final concentration of NADPH used, and the slope of this graph described the 'on-rate' of NADPH to the binary complex. Values for k⁻, i.e. the 'off-rate' of