Biosynthesis of *N*-(3-Aminopropyl)-1,4-diaminobutane (Spermidine) from L-Methionine in *Escherichia coli*: an Investigation by ^{13}C-Labelling

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Summary *E. coli* converts L-[3,4-$^{13}C_2$]methionine into *N*-3-aminopropyl)-1,4-diaminobutane labelled at C-3 and C-4 [for numbering see (**1**)], thus excluding azetidine as an intermediate in the reaction between 1,4-diaminobutane and decarboxylated adenosylmethionine catalysed by aminopropyl transferase.

THE polyamines 1,4-diaminobutane (putrescine), *N*-(3-aminopropyl)-1,4-diaminobutane (spermidine, **1**), and *NN'*-bis(3-aminopropyl)-1,4-diaminobutane (spermine, **2**) are of fundamental importance in cellular chemistry.[1] According to biosynthetic experiments[2] using radiolabelled compounds, 1,4-diaminobutane (from L-ornithine or L-arginine) and decarboxylated adenosylmethionine (**3**, from L-methionine) produce the polyamine (**1**) [or (**2**)] in a reaction catalysed by aminopropyl transferase.[3] As the first step to elucidating the mechanism of this reaction, we have investigated the biosynthesis of (**1**) from L-[3,4-$^{13}C_2$]-methionine by *E. coli*.

$$\mathrm{RNH\overset{9}{C}H_2\overset{8}{C}H_2\overset{7}{C}H_2\overset{6}{C}H_2NR\overset{4}{C}H_2\overset{3}{C}H_2\overset{2}{C}H_2NHR}$$

(**1**) R = H

(**5**) R = PhNHCS

$$\mathrm{H_2N[CH_2]_3NH[CH_2]_4NH[CH_2]_3NH_2}$$

(**2**)

$$\mathrm{Ad\overset{+}{S}(Me)\overset{1}{C}H_2\overset{2}{C}H_2\overset{3}{C}H_2NH_2}$$

(**3**) Ad = adenosyl

$$\mathrm{PhNHCSNH[CH_2]_4NHCSNHPh}$$

(**4**)

[1,2-$^{13}C_2$]Ethylene (B.O.C. Prochem Ltd., containing 81% $^{13}C_2$, 18% $^{12}C^{13}C$, and 1% $^{12}C_2$ species) was treated with methanesulphenyl chloride in CH_2Cl_2 (−30 °C, *ca.* 45 min) to give 1-chloro-2-(methylthio)-[1,2-$^{13}C_2$]ethane ([{^1H}^{13}C n.m.r. spectrum ([$^2H_6$]benzene): δ 35·7 (d, *J* 37 Hz, S*C*H_2) and 42·1 (d, *J* 37 Hz, *C*H_2Cl) p.p.m.], which was converted into DL-[3,4-$^{13}C_2$]methionine as described[4] for the unlabelled compound. This is the first synthesis of a methionine doubly labelled at C-3 and C-4 with ^{13}C and proceeds in 40% overall yield from ethylene. Resolution[5] of this methionine with ammonium (+)-α-bromocamphor-π-sulphonate gave L-[3,4-$^{13}C_2$]methionine.

Cells of an *E. coli* mutant were grown in a glucose-salts medium† supplemented with L-[3,4-$^{13}C_2$]methionine (0·035 g dm^{-3}). The cells (20 g wet weight) from 10 dm^3 growth media were washed with aqueous NaCl and KCl solutions and extracted with 0·4 mol dm^{-3} aqueous trichloroacetic acid. The resulting extract was washed with ether, made alkaline, and treated with ethanolic isothiocyanatobenzene (PhNCS) to give the *N'*-substituted *N*-phenylthioureas (**4**) and (**5**).‡ These derivatives were extracted with CH_2Cl_2 and were purified by preparative layer chromatography (Kieselgel 60 HR, double elution with 1:9 acetonitrile–CH_2Cl_2). As expected,[1,2] phenylthiourea (**4**) was not enriched with ^{13}C above natural abundance (n.m.r. analysis). The {^1H}^{13}C n.m.r. spectrum of authentic unlabelled (**5**) shows *inter alia* signals at δ ([$^2H_6$]dimethyl sulphoxide) 24·7 (C-7 or C-8), 26·1 (C-7 or C-8), 26·9 (C-3), 41·7 (C-2), 43·7 (C-9), 48·6 (C-4), and 50·2 (C-6) p.p.m. assigned by comparison with data for (**4**), the *N*-phenylthioureas of ethylamine and diethylamine, and the bis(*N*-phenylthiourea) of 1,3-diaminopropane. These assignments depend on shielding and deshielding effects similar to those observed with alcohols compared with ethers and primary amines compared with secondary amines.[6] The sample of (**5**) (65 mg) from L-[3,4-$^{13}C_2$]-methionine shows in its {^1H}^{13}C n.m.r. spectrum an intense AX system [doublets (*J* 35 Hz) astride singlets at δ 26·8 (C-3) and 48·5 (C-4) p.p.m.] in addition to signals at natural abundance. The intensities of the signals from C-3 and C-4 indicate that ^{13}C from methionine had been incorporated into (**1**) without significant dilution. This result proves that the C(3)–C(4) unit of methionine is the precursor of C(3)–C(4) of (**1**). Previous results[2] demonstrated that C-1 of methionine is lost *en route* to (**1**), whereas C-2 is retained.

There are three plausible alternative mechanisms for the reaction of 1,4-diaminobutane with (**3**) giving (**1**): (i) enzyme-mediated S_N2 attack of a nitrogen atom of 1,4-diaminobutane on C-1 of (**3**) leading directly to (**1**); (ii) S_N2 attack at C-1 of (**3**) by a nucleophilic group of aminopropyl transferase giving an aminopropylated enzyme which reacts with 1,4-diaminobutane, also by an S_N2 mechanism; (iii) enzyme-induced intramolecular closure of (**3**) to protonated azetidine which reacts with 1,4-diaminobutane.

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‡ A detailed account of these derivatives and related compounds, which can be used for quantitative analyses of polyamines in biological materials, will be published elsewhere.

Mechanism (iii) is excluded by the present results because the intermediacy of azetidine (homotopic C-2 and C-4) would have led to a 1 : 1 mixture of molecules of (**1**), either labelled at C-2 and C-3 or at C-3 and C-4.

Phenylthiourea (**5**) can be converted into (**1**) (hydrochloride) by refluxing overnight in conc. HCl, followed by evaporation and crystallisation of the residue from ethanol. Therefore [3,4-$^{13}C_2$]-(**1**) for studying the metabolism of (**1**)[7] and its interactions with nucleic acids[8] is now readily available.

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