SHORT COMMUNICATIONS

Origin of the D-I-Aminopropan-2-ol Fragment of Vitamin B₁₂

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I-Aminopropan-2-ol is a component of vitamin B₁₂ (Cooley, Ellis & Petrow, 1950) possessing the dg-configuration (Wolf, Jones, Valiant & Folkers, 1950). X-Ray crystallography confirmed that the amino alcohol linked the substituted corrin and nucleotide-like ring systems (Hodgkin *et al.* 1956; Hodgkin, 1958).

The metabolic origin of the nitrogen atom of D-I-aminopropan-2-ol in vitamin B₁₂ was established using [¹⁵N]-L-threonine, added to the growth medium of *Streptomyces griseus* (Krasna, Rosenblum & Sprinson, 1957). It was suggested that the carbon skeleton of the aminopropanol moiety was also derived from threonine by decarboxylation, although this was not investigated. Direct decarboxylation of the amino acid has never been demonstrated, and neither the threonine nor threonine-*O*-phosphate analogues of vitamin B₁₂ serve as precursors of cobinamide in either *Escherichia coli* or *Propionibacterium shermanii* (Bernhauer & Wagner, 1961, 1962). The discovery of L-threonine dehydrogenase (Neuberger & Tait, 1960, 1962) and D-I-aminopropan-2-ol dehydrogenase activity (Turner, 1966; Dekker & Swain, 1968; Lowe & Turner, 1968) revived interest in the possibility that the free amino alcohol, formed from L-threonine via aminoacetone, served as a precursor. The roles of L-threonine and D-I-aminopropan-2-ol in vitamin B₁₂ biosynthesis by a *Streptomyces* strain were therefore investigated using ¹⁴C-labelled compounds.

EXPERIMENTAL

The actinomycete used by Krasna *et al.* (1957) was not available and the alternative *Streptomyces olivaceus* NCIB 8238 was chosen in view of its previous use in studies of vitamin B₁₂ production (Hall, Benedict, Wiesen, Smith & Jackson, 1953; Weygand, Klebe & Trebst, 1954). Growth under conditions described by Hall *et al.* (1953) gave a yield of 0.5 mg. vitamin B₁₂/l. of entire culture in our hands. The vitamin was assayed microbiologically with the auxotrophic mutant *Escherichia coli* NCIB 8134 (Davis & Mingioli, 1950; Bessell, Harrison & Lees, 1950). L-Threonine (5.4 and 208 mCi/mmole) was obtained from the Radiochemical Centre, Amersham, Buckinghamshire. [3-¹⁴C]-DL-I-Aminopropan-2-ol hydrochloride (0.085 mCi/mmole) was kindly donated by Pfizer Ltd, Sandwich, Kent. [U-¹⁴C]-D-I-Aminopropan-2-ol hydrochloride was made from [U-¹⁴C]-L-threonine (208 mCi/mmole) by a modification of the thermal decarboxylation method of Chatelus (1964) (see Lowe & Turner, 1970). Radioactive D-I-aminopropan-2-ol was separated from unreacted L-threonine and non-radioactive phenylethylamine by thin-layer chromatography on cellulose using butan-1-ol + glacial acetic acid + water (12 + 3 + 5, by vol.). The *R_F* values were 0.39, 0.55 and 0.76

respectively. The radioactive amino alcohol also co-chromatographed with authentic D-1-aminopropan-2-ol as a single spot in ethanol + aqNH₃ (sp. gr. 0.880) + water (18 + 1 + 1, by vol.). The yield was about 16 %.

RESULTS

In initial experiments the uptake of radioactivity from [U-¹⁴C]-L-threonine (0.55 mg./l.; sp. act. 5.4 mCi/mmole) in growing cultures of *Streptomyces olivaceus* was complete and concomitant with the growth phase. Radioactivity within the micro-organism was mainly accounted for as threonine in cellular protein as shown by extraction and hydrolysis. Labelled 1-aminopropan-2-ol was not identified in any of the subcellular fractions, i.e. cold TCA, hot ethanol, hot TCA and hydrolysed protein (see Roberts, Abelson, Cowie, Bolton & Britten, 1957). In similar experiments radioactivity from [3-¹⁴C]-DL-1-aminopropan-2-ol (2.0 mg./l.; sp. act. 0.085 mCi/mmole) was found to be taken up only during the stationary phase, coinciding with the production of vitamin B₁₂. Chromatographic analysis of culture supernatants indicated complete uptake of DL-1-aminopropan-2-ol with the simultaneous excretion of several acidic compounds, the major one being identified as lactate. Analysis of radioactivity in subcellular fractions (see Roberts *et al.* 1957) did not show the presence of free labelled 1-aminopropan-2-ol or aminoacetone. Differences in the metabolism of L-threonine and DL-1-aminopropan-2-ol by *Streptomyces olivaceus* were further evident in studies using washed bacteria suspended in 0.1 M-phosphate buffer, pH 7. L-Threonine uptake (measured as the incorporation of radioactivity) occurred more rapidly when the actinomycete was harvested during the growth rather than stationary phase. Conversely, radioactivity from labelled DL-1-aminopropan-2-ol was only taken up by the micro-organism harvested in the stationary phase, and after a lag period of several hours.

To investigate the incorporation of radioactivity into the synthesized vitamin B₁₂ [U-¹⁴C]-L-threonine (0.5 μ mole; 100 μ Ci) was added to 6 l. of growth medium and the actinomycete cultured in 1000 ml. aliquots in 2 l. Ehrlenmeyer flasks at 28° for 5 days. Parallel experiments were carried out with an addition of [U-¹⁴C]-D-1-aminopropan-2-ol (0.1 μ mole; 16 μ Ci). Vitamin B₁₂ was extracted from the cultures as outlined in Table 1, the purified cyanocobalamin was hydrolysed and 1-aminopropan-2-ol isolated as described by Cooley, Ellis & Petrow (1950) and purified by thin-layer chromatography. From the data shown in Table 1 radioactivity from [U-¹⁴C]-L-threonine was specifically incorporated into the D-1-aminopropan-2-ol moiety of vitamin B₁₂. The ratio of radioactivity/carbon atom of D-1-aminopropan-2-ol to radioactivity/carbon atom of the remainder of the vitamin molecule was 12:1. Although the vitamin B₁₂ synthesized in the presence of [U-¹⁴C]-D-1-aminopropan-2-ol was radioactive, the amino alcohol released on hydrolysis had no measurable radioactivity and hence no specific incorporation of the amino alcohol had occurred. The analysis of radioactivity in other subcellular fractions (Roberts *et al.* 1957) of the micro-organism showed that D-1-aminopropan-2-ol had been incorporated into a wide range of substances, including lipids and proteins. Solvent extraction and chromatography, followed by hydrolysis of lipids and rechromatography, failed to show that the amino alcohol had been incorporated specifically into lipids, e.g. as a phosphatidyl base analogous to ethanolamine.

Table 1. *Incorporation of radioactivity from [U-¹⁴C]-L-threonine and [U-¹⁴C]-D-1-aminopropan-2-ol into the amino alcohol moiety of vitamin B₁₂*

The freeze-dried material from 6 l. of entire culture medium was suspended in 400 ml. water containing 2 mmoles KCN and autoclaved. The vitamin was extracted into 150 ml. *p*-cresol + carbon tetrachloride (1 + 4, wt/vol.) and back-extracted into 50 ml. of butan-1-ol + water (1 + 1, by vol.). Butan-1-ol was removed under reduced pressure and vitamin B₁₂ isolated from the aqueous solution by chromatography on the weak cation exchange resin Bio-Rex 70 as described by Strohecker & Henning (1965), followed by chromatography on DEAE cellulose from which it was eluted by 0.2 M-NaCl. The vitamin was finally purified by thin-layer chromatography on cellulose in the lower phase of butan-2-ol + methanol + sodium acetate buffer 0.2 M, pH 4.6 (4 + 1 + 12, by vol.), *R_F* 0.85. Vitamin B₁₂ was measured microbiologically as described in the text, and radioactivity was determined with a Nuclear Chicago gas flow counter.

Treatment	U-[¹⁴ C]-L-Threonine		[U- ¹⁴ C]-D-1-Aminopropan-2-ol	
	Total vitamin B ₁₂ (mg.)	Radioactivity (d.p.m.)	Total vitamin B ₁₂ (mg.)	Radioactivity (d.p.m.)
Culture medium	2.16	2.22 × 10 ⁸	1.62	3.56 × 10 ⁷
<i>p</i> -Cresol/carbon tetrachloride	1.97	—	1.46	—
Aqueous butan-1-ol	1.85	9.70 × 10 ⁵	1.39	4.20 × 10 ⁵
Bio-Rex 70	0.95	2.03 × 10 ⁵	0.70	1.09 × 10 ⁵
DEAE cellulose	0.65	1.07 × 10 ⁵	0.53	2.40 × 10 ⁴
Thin-layer chromatography	0.44	5.64 × 10 ⁴	0.36	1.23 × 10 ⁴
Hydrolyzed vitamin B ₁₂	0.31	3.96 × 10 ⁴	0.25	8.66 × 10 ³
D-1-Aminopropan-2-ol isolated from B ₁₂	—	1.36 × 10 ⁴	—	< 1.0 × 10 ³

DISCUSSION

The incorporation of isotope from [U-¹⁴C]-L-threonine into the 1-aminopropan-2-ol fragment of vitamin B₁₂ shows that the amino acid contributes the carbon skeleton, as well as the nitrogen atom (Krasna *et al.* 1957), of the amino alcohol. The failure to detect specific incorporation of isotope from [U-¹⁴C]-D-1-aminopropan-2-ol suggests that the free amino alcohol may not be involved in vitamin B₁₂ biosynthesis. The incorporation of radioactivity into a wide range of other substances, including lipids and proteins, confirmed that the D-enantiomorph is readily metabolized by *Streptomyces olivaceus* probably by way of aminoacetone (Lowe & Turner, 1970).

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