BIOSYNTHESIS OF 5, 6-DIMETHYLBENZIMIDAZOLE FROM RIBOFLAVIN

Transformation of C-1′ of riboflavin into C-2 of 5, 6-dimethylbenzimidazole

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1. Introduction

Recently it was found that riboflavin is transformed into the 5,6-DMBIA*-moiety of vitamin B_{12} by cell homogenates of *Propionibacterium shermanii* [1]. On the other hand Alworth et al. [2] showed in experiments with the same organism that with 1-^{14}C-ribose as substrate, 40% of the radioactivity incorporated into 5,6-DMBIA is found in the C-2 position.

In this publication experiments are described showing that riboflavin, which is ^{14}C-labelled at C-1′ of its ribityl side chain is transformed into 5,6-DMBIA exclusively labelled in its C-2 position.

2. Materials and methods

1-^{14}C-D-ribose (7.5 mCi/mmole) was purchased from Buchler a. Co., Braunschweig, Germany. Solvents for paper chromatography: I: butanol-2/water/acetic acid/10% aqueous KCN = 70/30/1/0.1. II: butanol-2/water/1% aqueous HCN = 70/29/0.5. Paper electrophoresis was performed in 0.5 M acetic acid (15 V/cm; 2–3 hr.). Riboflavin [3], vitamin B_{12}, α-ribazole and 5,6-DMBIA were determined spectrophotometrically [4]. Radioactivity was measured in a liquid scintillation counter (Beckman LS 150) with a solution of 6 g of PPO in 1 l of toluene and with internal standardization (^{14}C-toluene, Beckman).

2.1. Synthesis of 1′-^{14}C-riboflavin [5]

3,4-Dimethylaniline-D-riboside was prepared according to Berger et al. [6] and hydrogenated with the catalyst described by Kuhn et al [5] to 1-D-ribitylamino-3,4-dimethylbenzene as described by Shunk et al. [7] with the exception that the hydrogenation was performed at 35° and atmospheric pressure for 44 hr. 1-D-Ribitylamino-2phenylazo-4, 5-dimethylbenzene was prepared according to [7] and reacted with anhydrous barbituric acid according to a modification [8] of the method described by Tishler et al. [9].

From 220 mg of 3,4-dimethylaniline (1.82 mmole) and 200 mg of 1-^{14}C-D-ribose (1.33 mmole, 250 μCi) 91 mg (18% overall yield) of 1′-^{14}C-riboflavin (264,000 dpm/μmole) were obtained. The purity was checked after chromatography in KCN-free solvent I by radioactivity-scanning (Dünnschicht-scanner II, Fa. Berthold, Wildbad, Germany) and by detection of fluorescence under UV-light of 254 nm. No impurity could be detected. The 260/450 nm-ratio of a solution of 1′-^{14}C-riboflavin in 0.1 M phosphate buffer pH 7.0 was the same as that for pure riboflavin [3].

2.2. Growth of P. shermanii and incubation with 1′-^{14}C-riboflavin

P. shermanii St 33 was grown as described previously [10]. 35 g of wet cells from a 3 day-culture

* Abbreviations: 5,6-DMBIA = 5,6-dimethylbenzimidazole; α-ribazole = 5,6-dimethylbenzimidazole-α-D-ribofuranoside.

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were suspended in 400 ml of sterile 0.07 M phosphate buffer pH 7.0. After addition of 5 mg of 1′-^{14}C-riboflavin the mixture was incubated with continuous shaking (100 rpm, 40 hr, 25°). Vitamin B_{12} was isolated and purified on columns of Amberlite XAD-2, Dowex 2 × 8 and CM-cellulose as described previously [11].

The vitamin B_{12} thus obtained from 4 of the above incubation mixtures was further purified by descending chromatography on Whatman 3 MM-paper with solvent I. After rechromatography with solvent II the vitamin B_{12} was finally chromatographed on a column (1 × 10 cm) of Amberlite XAD-2 [11].

The α-ribazole obtained after cerous hydroxide degradation [10] of vitamin B_{12} was degraded to 5,6-DMBIA [12]. The 5,6-DMBIA was purified by paper electrophoresis in 0.5 M acetic acid and eluted from the paper with 70% aqueous ethanol. Degradation of 5,6-DMBIA to 1,2-dibenzamido-4,5-dimethylbenzene and formic acid [13], as well as the oxidation of the formic acid to CO_2, was performed as described by Alworth et al. [14]. CO_2 was absorbed in 2-phenylethylamine [15] in the absorption device of the decarboxylation apparatus described by Phares [16].

3. Results and discussion

In table 1 the data are given which demonstrate that radioactivity from 1′-^{14}C-riboflavin is incorporated into the 5,6-DMBIA-moiety of vitamin B_{12} in *P. shermanii*. From these data it can be calculated that 10% of the 1′-^{14}C-riboflavin added to the bacteria was transformed into 5,6-DMBIA. It is noteworthy that the specific radioactivity of riboflavin and of vitamin B_{12} differ only slightly. This means that the amount of endogenous riboflavin competing with the radioactive riboflavin does not exceed 10%. Table 2 shows that the total amount of radioactivity of 5,6-DMBIA is localized in the C-2 position. This proves that C-2 of 5,6-DMBIA is derived from C-1′ of riboflavin.

Table 1
Incorporation of radioactivity from 1′-^{14}C-riboflavin into the 5,6-DMBIA-moiety of vitamin B_{12}* by *P. shermanii*.

	Amount (mg)	Specific radioactivity (dpm/μmole)
1′-^{14}C-riboflavin	20	264,000
Vitamin B_{12}		
1. After paper chromatography in solvent I	7.6	234,500
2. After paper chromatography in solvent II	6.15	227,500
3. After chromatography on Amberlite XAD-2	5.95	249,000
Products of cerous hydroxide degradation of 4.1 mg vitamin B_{12}		
Cobinamide	2.06	810
α-Ribazole	0.57	212,500
5,6-DMBIA		
1. From HCl-degradation of α-ribazole	0.26	205,500
2. After paper electrophoresis in 0.5 M acetic acid	0.23	225,000

* 4 of the incubation mixtures described in Materials and methods were run. For the isolation of vitamin B_{12} the bacteria were pooled. led.

Table 2
Evidence for the localization of the radioactivity of 1′-^{14}C-riboflavin in C-2 of 5,6-DMBIA.*

	Amount (mg)	Total dpm found	dpm/μmole
5,6-DMBIA**	29.23	354,000	1,770
1,2-dibenzamido-4,5-dimethylbenzene	38.4	2,520	23
CO_2 from C-2 of 5,6-DMBIA	–	201,400	1,675 †

* For experimental details see Materials and methods.
** For treatment with benzoyl chloride the 5,6-DMBIA mentioned in table 1 was diluted with 29 mg of nonradioactive 5,6-DMBIA.
† In several experiments with nonradioactive material it was found that the average yield of CO_2 from 5,6-DMBIA was 60% determined as $BaCO_3$. This value was used to calculate the specific radioactivity of CO_2, since the amount of CO_2 was not determined in the experiment with radioactive material.

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