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RIBITOL-^{14}C INCORPORATION IN THE RIBITYL SIDE CHAIN OF RIBOFLAVIN

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OUR KNOWLEDGE of the biosynthetic path to riboflavin has increased considerably during recent years, but some questions need further clarification. It is well established that riboflavin formation starts from a purine,[1] but less is known about the origin of the ribityl side chain. There is no evidence for the reduction of a pyrimidylaminopentulose[2] which would arise from a purine nucleoside by elimination of carbon-8.

This paper describes the incorporation of both ribose-U-^{14}C and ribitol-U-^{14}C into vitamin in the low riboflavin overproducer *Candida guilliermondii*. From a comparative viewpoint, guanosine-U-^{14}C and adenosine-U-^{14}C have been applied to the yeast, too. The labelled compounds were fed over 4- to 20-hr-old cultures of *Candida* grown either under iron deficiency[3] or in presence of 500 μg Fe^{2+}/l. culture medium. Iron was applied as a complex of the ferrous ion with diaminoethane-tetraacetic acid. The riboflavin overproduction is bound to iron deficient conditions, whereas in iron containing media the overproduction is repressed.

Radioactive riboflavin was isolated as follows: The culture broth was centrifuged, the precipitated cells were washed with 50 ml 5% EtOH 3 times and the combined supernatants were desalted using a Sephadex G15 column. This material was fractionated with twice distilled water using polyvinylpyrrolidone and a Sephadex G15 column. The purity of isolated riboflavin was proved by UV and fluorescence spectroscopy, by TLC in 4 solvents (H_2O, *n*-BuOH–HOAc–H_2O, 4:1:5, *n*-BuOH-5N HOAc, 2:1, MeOH), by electrophoresis on cellogel at pH 8·6 (0·1 M veronal buffer) and pH 5·1 (0·05 M HOAc–NaOH buffer), and by comparison of K_{av} values of isolated and authentic compound. Purified riboflavin was free from lumichrome. After application of carbon-labelled ribose and nucleosides, the specific incorporation rates in riboflavin are relatively low (0·008% with guanosine, 0·013% with adenosine and 0·03% with ribose) and the introduction of radiocarbon from these compounds in the vitamin is rather unimportant in iron deficient cells. These results obtained with nucleosides containing only about 73% of total radioactivity in the ribose moiety correspond to data of McNutt.[4] The pool of free nucleosides in *Candida* cells is extremely low. Therefore, practically no dilution of exogenously supplied compounds by endogenous nucleosides

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occurred. Under iron deficiency, ribitol-U-^{14}C was introduced in riboflavin with a specific incorporation rate of 0·44%. Surprisingly, there is a remarkably large amount of free ribitol in iron deficient cells (about 1·5% on dry wt). The polyol was isolated and identified by PC and m.p.[5] This finding might explain the relatively low incorporation of ribitol-U-^{14}C in riboflavin when compared with the high value of the specific incorporation rate in cells grown in the presence of 500 μg Fe^{2+}/l. culture medium, namely 7·7%. The ribitol pool is rather unimportant for iron grown cells.

We assume therefore that ribose and nucleosides play no direct role in riboflavin biosynthesis, but ribose may be precursor of ribitol. Ribitol seems to be an important source of the ribityl group of riboflavin. This idea is favoured by data obtained from the degradation of labelled riboflavin formed after ribitol-U-^{14}C feedings (Table 1). Samples of labelled riboflavin were illuminated with UV light in methanolic solution.[6] The lumichrome formed was separated from undecomposed riboflavin by PC with twice distilled water. Radioactive measurements were made by means of a Tricarb scintillation counter. The distribution of radiocarbon in the isoalloxazine nucleus and the ribityl side chain was estimated by determination of the radioactivity of the intact riboflavin and lumichrome. From these data the introduction of radioacrbon in the ribityl side group was calculated. The results obtained fairly well agree with our findings that adenine-2-^{14}C and guanine-2-^{14}C were exclusively incorporated into the isoalloxazine nucleus of riboflavin.

TABLE 1. DISTRIBUTION OF RADIOCARBON IN THE RIBOFLAVIN MOLECULE AFTER FEEDING RIBOSE-U-^{14}C AND RIBITOL-U-^{14}C TO *Candida* CELLS GROWN UNDER DIFFERENT CULTURE CONDITIONS

Precursor	Ribose-U-^{14}C		Ribitol-U-^{14}C	
Iron conc. (μg Fe^{2+}/l.)	< 10	500	< 10	500
Spec. incorporation rate in riboflavin (%)	0·03	0·09	0·44	7·74
Spec. radioactivity (mCi/mmol)				
riboflavin (isolated)	0·029	0·087	0·008	0·141
lumichrome (from riboflavin)	0	0·002	0	0·004
Percentage of radiocarbon in				
isoalloxazine nucleus (estimated)	0	2	0	3
ribityl side chain (calculated)	100	98	100	97

By analogy to cell wall formation in gram positive bacteria, ribitol in riboflavin biosynthesis might be metabolized via ribitol phosphate and CDP-ribitol.[7] Further work on this pathway is in progress.

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