BIOSYNTHESIS OF RIBOFLAVIN. MUTANTS ACCUMULATING 6-HYDROXY-2,4,5-TRIAMINOPYRIMIDINE

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1. Introduction

The present knowledge of the biosynthesis of riboflavin (5) is outlined in fig. 1. The direct precursor of riboflavin, 6,7-dimethyl-8-ribityllumazine (4), was isolated from *Eremothecium ashbyii* [1] and from a number of other microorganisms [2,3]. 5-Amino-2,6-dihydroxy-4-ribitylaminopyrimidine (3) and 2,5-diamino-6-hydroxy-4-ribitylaminopyrimidine (2) were detected in the culture media of riboflavin auxotrophs of *Saccharomyces cerevisiae* [3,4]. The involvement of a purine precursor in riboflavin biosynthesis has been demonstrated repeatedly [5,6]. Recently Bacher and Lingens have demonstrated by experiments with a purine auxotroph of *Aerobacter aerogenes*, that a purine compound at the biosynthetic level of guanine (1) is used [7]. Baugh and Krumdieck obtained similar results with a wild strain of *Corynebacterium* sp. inhibited by decoyinine [8].

The present paper is concerned with the detection of 6-hydroxy-2,4,5-triaminopyrimidine (6) in riboflavin auxotrophs of *S. cerevisiae* and with the complementation behaviour of these mutants.

2. Materials and methods

2-Amino-6,7-dimethyl-4-hydroxypteridine was a gift of Prof. Pfleiderer, Konstanz. 2-Amino-4-hydroxypteridine was synthesized [9].

Riboflavin auxotrophs *N* 179 and *N* 213 were isolated after treatment of *S. cerevisiae S* 288 *C* with 1-methyl-3-nitro-l-nitrosoguanidine essentially as described by Oltmanns and Lingens [10].

S. cerevisiae N 179 was grown in a 100 l batch fermentor in minimal medium [10] supplemented with yeast extract (Difco) (1.5 g/l), peptone (Difco) (1.5 g/l) and riboflavin (10 mg/l). The cells were harvested and resuspended in minimal medium supplemented with diacetyl (0.8 g/l) or glyoxal (0.6 g/l). After 24 hr, the suspension was centrifuged. The supernatant was passed through a column of magnesium silicate (Woelm). The column was washed with water and eluted with acetone - 2 N ammonia (2:1, v/v). The eluate

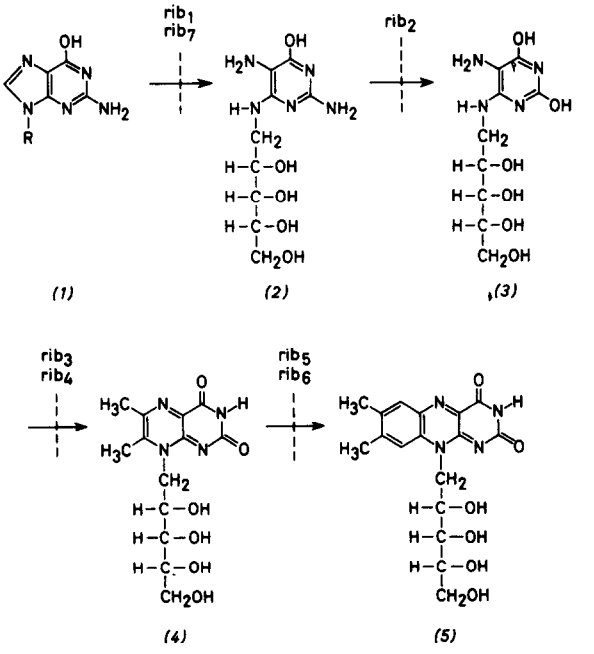

Fig. 1. Biosynthesis of riboflavin and genetic blocks of *S. cerevisiae* mutants.

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was evaporated to dryness *in vacuo*. From the residue pteridines were isolated by chromatography on Dowex 50 W X 8 (elution with HCl). The compounds were recrystallized from water.

Complementation tests were performed as described elsewhere [11].

3. Results

We have found previously that the identification of pyrimidine intermediates of riboflavin biosynthesis is greatly simplified by condensation of these labile substances with glyoxal or diacetyl prior to isolation [3,4]. By application of this technique we now were able to demonstrate the formation of 6-hydroxy-2,4,5-triaminopyrimidine (6) in two riboflavin auxotrophs.

A blue fluorescent compound is formed when mutant *N* 179 is incubated in minimal medium containing diacetyl. This compound was isolated as described in Methods. We obtained 1.5 mg of colourless microcrystals from 100 l of medium. They could be identified as 2-amino-6,7-dimethyl-4-hydroxypteridine (7) by means of UV- and IR-spectrometry and by thin-layer chromatography in 5 solvent systems.

In a similar experiment, glyoxal was added to the accumulation medium instead of diacetyl. We isolated 2-amino-4-hydroxypteridine (8) (0.4 mg/100 l), which was identified by the same methods.

None of the pteridines described could be isolated from fermentations without either glyoxal or diacetyl. We conclude from these results, that the mutant produces 6-hydroxy-2,4,5-triaminopyrimidine (6) (fig. 2). Experiments with mutant *N* 213 gave the same results.

In previous experiments a minimum number of 6 riboflavin genes (rib_1 - rib_6, corresponding to complementation groups I-VI) was detected in *S. cerevisiae* [11] (see fig. 1). We found that mutants *N* 179 and *N* 213 complement with mutants of complementation groups I-V (table 1). We have not tested complementation group VI (accumulating 6,7-dimethyl-8-ribityllumazine) for technical reasons. Probably the mutants *N* 179 and *N* 213 (complementation group VII) represent a new gene (rib_7) rather than a case of intragenic complementation.

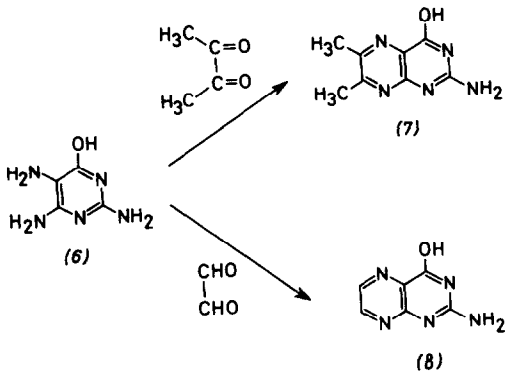

Fig. 2. Condensation of 6-hydroxy-2,4,5-triaminopyrimidine with diacetyl and glyoxal, respectively.

Table 1
Complementation of riboflavin auxotrophs of *S. cerevisiae*; C.G. = complementation group, + = growth of diploids on minimal medium, s = slow growth.

Gene		rib_7		
	C.G.	VII		
		mutant	N 179	N 213
rib_1	I	HK 761	+	s
rib_1	I	HK 851	s	+
rib_2	II	HK 857	+	+
rib_3	III	HK 693	+	+
rib_3	III	HK 882	s	+
rib_4	IV	HK 829	+	+
rib_4	IV	HK 871	s	+
rib_4	IV	HK 890	+	+
rib_5	V	HK 750	s	+
rib_5	V	HK 873	+	+

4. Discussion

From the formation of 2,5-diamino-6-hydroxy-4-ribitylaminopyrimidine (2) in riboflavin auxotrophs of *S. cerevisiae*, Bacher and Lingens concluded that the biosynthesis of riboflavin starts from a derivative of guanine rather than from another purine compound [4]. This hypothesis was confirmed by studies on a purine auxotroph of *A. aerogenes* [7]. It receives further

support by the detection of 6-hydroxy-2,4,5-triaminopyrimidine in riboflavin auxotrophs of *S. cerevisiae*.

The mutants described in this paper are probably defective in the conversion of the guanine precursor (1) to 2,5-diamino-6-hydroxy-4-ribitylaminopyrimidine (2). The question still remains, whether 6-hydroxy-2,4,5-triaminopyrimidine is a true intermediate of riboflavin biosynthesis or a secondary product originating from the decomposition of an intermediate, especially by loss of a ribose moiety. In particular, our findings do not exclude the possibility, that the biosynthesis of 2,5-diamino-6-hydroxy-4-ribitylaminopyrimidine proceeds via the same intermediates as the biosynthesis of dihydroneopterinephosphate [12].

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