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Advance in Riboflavin Biosynthesis: Fermentation Techniques, Enzymatic Pathways, Utilisation and Industrial Applications

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Abstract:
Two molecules of ribulose 5-phosphate and one molecule of GTP are needed as substrates for the production of one riboflavin molecule. GTP's imidazole ring is hydrolytically opened to produce a 4,5-diaminopyrimidine, which is then followed by deamination, side chain reduction, and dephosphorylation to produce 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione. 6,7-dimethyl-8-ribityllumazine is produced by condensing 5-amino-6-ribitylamino2,4(1H,3H)-pyrimidinedione with 3,4-dihydroxy-2-butanone 4-phosphate, which is derived from ribulose 5-phosphate. Riboflavin and 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione are produced upon dismutation of the lumazine derivative and are then recycled throughout the biosynthetic process. Many studies have been conducted on the structure of the biosynthetic enzyme 6,7-dimethyl-8-ribityllumazine synthase. Under the direction of the inducible Pxyl promoter in B. subtilis PY, overexpression of glucose-6-phosphate dehydrogenase (G6PDH) altered carbon flow in Bacillus subtilis through the pentose phosphate (PP) pathway. The yield of riboflavin and the availability of ribulose-5-phosphate (Ru5P) will change if the carbon input into the PP pathway is altered. The specific growth rate remained constant, but the glucose consumption rate significantly increased as a result of overexpression of G6PDH. A 25%±2 increase in riboflavin synthesis was achieved. Lower acid production (acetate andpyruvate) and higher acetoin formation were noted in comparison to by-product formation in flask culture. The results of metabolic studies and carbon flux redistribution showed that overexpression of G6PDH increases the fluxes in the PP pathway.

Keywords: riboflavin, GTP Cyclohydrolase, Riboflavin Synthase, Lumazine Synthase, Ribulose-5-phosphate, Bacillus subtilis.

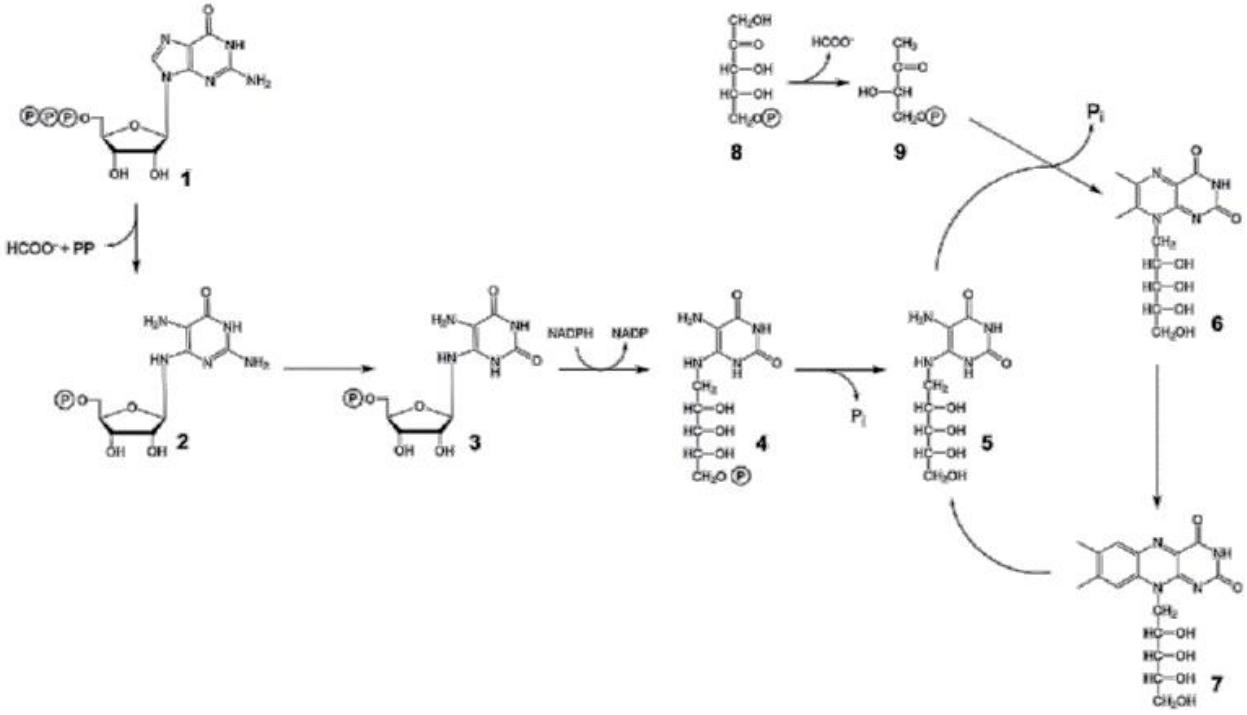
Introduction:

Many microorganisms and plants both biosynthesize riboflavin, or vitamin B2. The two main food sources of vitamin A for humans are vegetables and milk. The daily recommendation of vitamin B2 is 1.8mg. Riboflavin, also known as vitamin B2, is an essential micronutrient required for various cellular processes in humans and animals. While it can be obtained from dietary sources, riboflavin fermentation has emerged as a cost-effective and sustainable method for large-scale production. This article delves into the intricate process of riboflavin fermentation, the key enzymes involved, and the diverse applications of this vitamin. summarizes the pathway involved in biosynthesis. GTP cyclohydrolase II catalyzes the hydrolytic opening of the imidazole ring of GTP under the release of formate and pyrophosphate In two reaction steps, the hydrolytic cleavage of the position 2 amino group of the heterocyclic ring and the reduction of

the ribosyl side chain, which yields the ribityl side chain of the vitamin, transform the enzyme product 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 50-phosphate into 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione 50-phosphate . Different species go through these chemical steps in different orders. In eubacteria, side chain reduction comes before deamination.

The dismutation of 6,7-dimethyl8-ribityllumazine , which is mediated by riboflavin synthase, is the last step in the biosynthetic route.

5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione is the second result of the dismutation. This substance is recycled in the biosynthesis pathway and is a substrate of lumazine synthase. According to stoichiometry, two equivalents of ribulose 5-phosphate and one equivalent of GTP are needed to form one riboflavin. Therefore, all but four of the riboflavin molecule's 17 carbon atoms come from the pentose phosphate pool.



Riboflavin Fermentation Process:

Riboflavin fermentation involves the microbial synthesis of riboflavin from simple substrates such as glucose or other carbohydrates. The process typically employs microorganisms such as Ashbya gossypii, Bacillus subtilis, or genetically modified strains of Escherichia coli.

The fermentation process consists of several stages:

1. **Substrate Preparation**: Carbohydrate sources like glucose, sucrose, or molasses are prepared as the primary substrates for microbial growth and riboflavin production.
2. **Inoculum Development**: A starter culture of the selected microorganism is grown under controlled conditions to achieve optimal cell density and metabolic activity.
3. **Fermentation**: The inoculum is introduced into a larger fermentation vessel containing the substrate medium. Conditions such as pH, temperature, oxygen levels, and nutrient concentrations are carefully controlled to promote microbial growth and riboflavin synthesis.
4. **Harvesting**: After the fermentation process is complete, the broth containing riboflavin is harvested from the fermentation vessel.
5. **Purification**: The harvested broth undergoes purification steps to isolate and concentrate riboflavin

from other fermentation by-products and impurities.

Biosynthesis of Riboflavin:

A technical bulk product used in animal and human nutrition is riboflavin. The vitamin was mostly made by chemical synthesis until recently. Currently, the synthetic synthesis approach is gradually being replaced by fermentation processes employing B. subtilis, Ashbya gossypii, or Candida yeasts. The synthesis of vitamin B2 using recombinant B. subtilis strains has been extensively documented in other sources.

Actinobacillus pleuropneumoniae mutants lacking in riboflavin have been suggested as a potential vaccination for pigs. Enterobacteriaceae lack a riboflavin absorption mechanism. As a result, whereas riboflavin-deficient mutants can be grown in vitro in the presence of high riboflavin concentrations, they are unable to thrive in a mammalian host.

Key Enzymes Involved:

Several enzymes play crucial roles in riboflavin biosynthesis during fermentation. Some of the key enzymes include:

1. **GTP Cyclohydrolase II (RibA):** Catalyzes the conversion of GTP (guanosine triphosphate) to 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate (DARPP), an early intermediate in the riboflavin biosynthetic pathway.
2. **Riboflavin Synthase (RibC):** Mediates the final step in riboflavin biosynthesis by condensing two molecules of 6,7-dimethyl-8-ribityllumazine to form riboflavin.
3. **Lumazine Synthase (RibH):** Catalyzes the formation of 6,7-dimethyl-8-ribityllumazine, an intermediate in the riboflavin biosynthetic pathway.

Applications of Riboflavin:

Riboflavin finds wide-ranging applications across various industries, including:

- **Food and Beverage**: Riboflavin is used as a food additive and nutritional supplement in products such as breakfast cereals, energy drinks, and fortified dairy products.
- **Pharmaceuticals**: Riboflavin supplements are prescribed to treat deficiencies and certain medical conditions. It is also used as an ingredient in skincare products and cosmetics.
- **Animal Feed**: Riboflavin is added to animal feed formulations to improve livestock health and productivity.
- **Biotechnology:** Riboflavin serves as a precursor for the synthesis of flavin cofactors, which are essential for numerous enzymatic reactions in biotechnological processes.

In conclusion, riboflavin fermentation represents a sustainable and economically viable approach for the large-scale production of this essential vitamin. Understanding the microbial synthesis process, key enzymes involved, and diverse applications of riboflavin underscores its significance in various industries and human health.

References:

1. Ajayi OA, James OA. 1984. Effect of riboflavin supplementation and riboflavin nutriture of a secondary school population in Nigeria. Am. J. Clin. Nutr. 39:787–91.
2. Bacher A. 1991. Biosynthesis of flavins. See Ref. 73a, 1:215–59.

3. Bacher A, Baur R, Eggers U, Harders H, Otto MK, Schnepple H. 1980. Riboflavin synthases of Bacillus subtilis. Purification and properties. J. Biol. Chem. 255:632– 37.
4. Bacher A, Eberhardt S, Richter G. 1996. Biosynthesis of riboflavin. In Escherichia coli and Salmonella, ed. FC Neidhardt, pp. 657–64. Washington, DC: Am. Soc. Microbiol
5. Bacher A, Eisenreich W, Kis K, Ladenstein R, Richter G, et al. 1993. Biosynthesis of flavins. In Bioorganic Chemistry Frontiers, ed. H Dugas, FP Schmidtchen, pp. 147–92. New York: Springer Verlag.
6. Bacher A, Eisenreich W, Kis K, Richter G, Scheuring J, Weinkauf S. 1993. Recent advances in the biosynthesis of flavins and deazaflavins. Trends Org. Chem. 4:335–49.
7. Bacher A, Ladenstein R. 1991. The lumazine synthase/riboflavin synthase complex of Bacillus subtilis. See Ref. 73a, 1:293– 316.
8. Bacher A, Le Van Q, Keller PJ, Floss HG. 1983. Biosynthesis of riboflavin. Incorporation of 13C labelled precursors into the xylene ring. J. Biol. Chem. 258:13431–37.
9. Bacher A, Le Van Q, Keller PJ, Floss HG. 1985. Biosynthesis of riboflavin. Incorporation of multiply 13C-labeled precursors into the xylene ring. J. Am. Chem. Soc. 107:6380–85.
10. Bacher A, Lingens F. 1970. Biosynthesis of riboflavin. Formation of 2,5-diamino-6- hydroxy-4-(10 -D-ribitylamino)pyrimidine in a riboflavin auxotroph. J. Biol. Chem. 245:4647–52.
11. Beach R, Plaut GWE. 1970. Investigations of structures of substituted lumazines by deuterium exchange and nuclear magnetic resonance spectroscopy. Biochemistry 9:760–70.
12. Bown DH, Keller PJ, Floss HG, Sedlmaier H, Bacher A. 1986. Solution structure of 6,7-dimethyl-8-substituted lumazines. 13C NMR evidence for intramolecular ether formation. J. Org. Chem. 51:2461–67.
13. Bresler SE, Cherepenko EI, Chernik TP, Kalinin VL, Perumov DA. 1970. Investigation of the operon of riboflavin synthesis in Bacillus subtilis. I. Genetic mapping of the linkage group. Genetika 6:116–24.
14. Bresler SE, Cherepenko EI, Perumov DA. 1970. Investigation of the operon of riboflavin synthesis in Bacillus subtilis. II. Biochemical study of regulator mutations. Genetika 6:126–39.
15. Bresler SE, Cherepenko EI, Perumov DA. 1971. Investigation of the operon of riboflavina biosynthesis in Bacillus subtilis. III. Production and properties of mutants with a complex regulatory genotype. Genetika 7:117–23.
16. Barbe V, Cruveiller S, Kunst F, Lenoble P, Meurice G, Sekowska A, Vallenet D, Wang T, Moszer I, Medigue C, Danchin A (2009) From a consortium sequence to a unified sequence: the Bacillus subtilis 168 reference genome a decade later. Microbiology 155(Pt 6):1758–1775.
17. Becker J, Klopprogge C, Zelder O, Heinzle E, Wittmann C (2005) Amplified expression of fructose 1,6-bisphosphatase in Corynebacterium glutamicum increases in vivo flux through the pentose phosphate pathway and lysine production on different carbon sources. Appl Environ Microbiol 71(12):8587–8596.
18. Becker J, Klopprogge C, Herold A, Zelder O, Bolten CJ, Wittmann C (2007) Metabolic flux engineering of L-lysine production in Corynebacterium glutamicum-over expression and modification of G6P dehydrogenase. J Biotechnol 132(2):99–109 .
19. Blencke HM, Homuth G, Ludwig H, Mader U, Hecker M, Stulke J (2003) Transcriptional profiling of gene expression in response to glucose in Bacillus subtilis: regulation of the central metabolic pathways. Metab Eng 5(2):133–149.

20. Coquard D, Huecas M, Ott M, van Dijl JM, van Loon AP, Hohmann HP (1997) Molecular cloning and characterisation of the ribC gene from Bacillus subtilis: a point mutation in ribC results in riboflavin overproduction. Mol Gen Genet 254(1):81–84.
21. Hemberger S, Pedrolli DB, Stolz J, Vogl C, Lehmann M, Mack M (2011) RibM from Streptomyces davawensis is a riboflavin/ roseoflavin transporter and may be useful for the optimization of riboflavin production strains. BMC Biotechnol 11:119.
22. Humbelin M, Griesser V, Keller T, Schurter W, Haiker M, Hohmann HP, Ritz H, Richter G, Bacher A, van Loon APGM (1999) GTP cyclohydrolase II and 3,4-dihydroxy-2-butanone 4-phosphate synthase are rate-limiting enzymes in riboflavin synthesis of an industrial Bacillus subtilis strain used for riboflavin production. J Ind Microbiol Biotechnol 22(1):1–7.
23. Jules M, Le Chat L, Aymerich S, Le Coq D (2009) The Bacillus subtilis ywjI (glpX) gene encodes a class II fructose-1,6- bisphosphatase, functionally equivalent to the class III Fbp enzyme. J Bacteriol 191(9):3168–3171.
24. Kalingan AE, Liao CM (2002) Influence of type and concentration of flavinogenic factors on production of riboflavin by Eremothecium ashbyii NRRL 1363. Bioresour Technol 82(3):219–224.
25. Geckil H, Barak Z, Chipmannn DM, Erenler SO, Webster DA, Stark BC (2004) Enhanced production of acetoin and butanediol in recombinant Enterobacter aerogenes carrying Vitreoscilla hemoglobin gene. Bioprocess Biosys Eng 26:325–330 .
26. Goel A, Ferrance J, Jeong J, Ataai MM (1993) Analysis of metabolic fluxes in batch and continuous cultures of Bacillus subtilis. Biotechnol Bioeng 42:686–696 .
27. Grundy FJ, Waters DA, Takova TY, Henkin TM (1993) Indetification of genes involved in utilization of acetate and acetoin in Bacillus subtilis. Mol Microbiol 10:259–271.
28. Horne RN, Anderson WB, Nordlie RC (1970) Glucose dehydrogenase activity of yeast glucose-6-phosphate dehydrogenase. Inhibition by adenosine 5′-triphosphate and other nucleoside 5′-triphosphates and diphosphates. Biochemistry 9(3):610–616.
29. Hümbelin M, Griesser V, Keller T, Schurter W, Haiker M, Hohmann HP, Ritz H, Richter G, Bacher A (1999) GTP cyclohydrolase II and 3, 4-dihydroxy-2-butanone 4-phosphate synthase are ratelimiting enzymes in riboflavin synthesis of an industrial Bacillus subtilis strain used for riboflavin production. J Ind Microbiol Biotechnol 22:1–7.
30. Koizumi S, Yonetani Y, Maruyama A, Teshiba S (2000) Production of riboflavin by metabolically engineered orynebacterium ammoniagenes. Appl Microbiol Biotechnol 53:674–679.
31. Li XJ, Chen T, Chen X, Zhao XM (2006) Redirection electron flow to high coupling efficiency of terminal oxidase to enhance riboflavin biosynthesis. Appl Microbiol Biotechnol 73:374–383.
32. Lim SJ, Jung YM, Shin HD, Lee YH (2002) Amplification of the NADPH-Related genes zwf and gnd for the oddball biosynthesis of PHB in an E.coli thansformant harboring a cloned phbCAB operon. J Biosci Bioeng 93:543–549.
33. MacLaren JA. 1952. The effects of certain purines and pyrimidines upon the production of riboflavin by Eremothecium ashbyii. J. Bacteriol. 63:233–41.
34. Mail¨ander B, Bacher A. 1976. Biosynthesis of riboflavin. Structure of the purine precursor and origin of the ribityl side chain. J. Biol. Chem. 251:3623–28..
35. Masuda T. 1956. Application of chromatography. XXIX. G compound isolated from the mycelium of Eremothecium ashbyii. Pharm. Bull. 4:375–81.

36. Masuda T. 1956. Isolation of a green fluorescent substance produced by Eremothecium ashbyii. Pharm. Bull. 4:71–72.
37. Koltun LV, Shavlovsky GM, Kashchenko VE, Trach VM. 1984. Changes in the activity of certain enzymes involved in flavinogenesis during cultivation of Eremothecium ashbyii. Microbiology 53:32–36.
38. Kraut H. 1960. Uberdie Deckung des N¨ ¨ ahrstoffbedarfs in Westdeutschland. Nutr. Dieta 1:45–60.
39. Kugelbrey K. 1997. Biosynthese von Vitamin B2. NMR-Untersuchungen zum Reaktionsmechanismus der 6,7-Dimethyl8-ribityllumazin-Synthase. PhD thesis. Technische Universit¨at, M¨unchen. 197 pp.
40. Harzer G, Rokos H, Otto MK, Bacher A, Ghisla S. 1978. Biosynthesis of riboflavin. 6,7-Dimethyl-8-ribityllumazine 50 -phosphate is not a substrate for riboflavin synthase. Biochim. Biophys. Acta 540:48–54.