BIOSYNTHESIS OF RIBOSE AND DEOXYRIBOSE IN PSEUDOMONAS SACCHAROPHILA

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ABSTRACT

Fossitt, Dexter D. (University of Michigan, Ann Arbor), and I. A. Bernstein. Biosynthesis of ribose and deoxyribose in Pseudomonas saccharophila, J. Bacteriol. 86:1326–1331. 1963.—The biosynthesis of ribose and deoxyribose in Pseudomonas saccharophila was studied by radioisotope-tracer techniques. Patterns of C\textsuperscript{14} in ribose isolated from the nucleic acids of cells grown on labeled glucose suggested that pentose was made by the pathway involving transaldolase and transketolase. When cells were grown on radioactive gluconate, the tracer patterns indicated the possibility of a new pathway for the biosynthesis of ribose. Isotopic patterns in deoxyribose, in general, were consistent with the pathway involving reduction of ribonucleotides to deoxyribonucleotides. Certain aspects of the data, however, were not explained by this known pathway.

Pseudomonas saccharophila (Doudoroff, 1940) presents a unique system for the study of the biosynthesis of ribose and deoxyribose from glucose by radioisotope techniques, in that oxidation of glucose occurs by the Entner-Doudoroff pathway (Entner and Doudoroff, 1952) to the exclusion of the “hexose-monophosphate shunt” and the classical Embden-Meyerhof pathways. In such a system, interpretations of isotopic patterns in pentoses are not subject to numerous contributions from metabolic precursors formed from different pathways of carbohydrate utilization. The experiments included here were performed with P. saccharophila, since this organism has these unique characteristics, and since a detailed investigation of the biosynthesis of ribose and deoxyribose had not previously been made in this bacterium.

The two major pathways for the synthesis of ribose which were investigated in these experiments were (i) via the oxidative decarboxylation of 6-phosphogluconate, a sequence of reactions found in numerous biological systems (Horecker and Smyrniotis, 1951; Lanning and Cohen, 1954; Bernstein, 1956), and (ii) via a series of reactions catalyzed by transketolase and transaldolase (De la Haba and Racker, 1952; Horecker and Smyrniotis, 1952; Bernstein, 1953a; Bloom, Stetten, and Stetten, 1953; Hiatt and Lareau, 1960). The results of isotopic data in these experiments indicate that the nonoxidative pathway utilizing reactions, catalyzed by transketolase and transaldolase, could be the major route for the biosynthesis of ribose in P. saccharophila.

Two possible pathways for the biosynthesis of deoxyribose have been proposed: condensation of acetaldehyde and glyceraldehyde-3-phosphate (Racker, 1952), and direct reduction of ribose at carbon 2 while the pentose is in the form of a nucleotide diphosphate (Reichard and Rutberg, 1960; Bertani, Haggmark, and Reichard, 1961). Recently, Ghosh and Bernstein (1963) presented data not in agreement with the biosynthesis of deoxyribose by either “direct reduction” of ribonucleotides or by condensation of acetaldehyde and glyceraldehyde-3-phosphate; these data may indicate a third possible pathway for biosynthesis of deoxyribose. The studies reported here indicate that most of the deoxyribose is synthesized by the reduction of ribonucleotides to the corresponding deoxyribonucleotides.

1 This paper is taken from a dissertation submitted by Dexter D. Fossitt to the Graduate School of The University of Michigan in partial fulfillment of the requirements for the Ph.D. degree. A preliminary account of this work was presented at the 60th Annual Meeting of the Society of American Bacteriologists, Philadelphia, Pa., May, 1960.

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Materials and Methods

*P. saccharophila* was grown on a liquid mineral medium supplemented with 0.25% glucose or gluconate (Wiame and Doudoroff, 1951) for 30 hr at 30 C with vigorous aeration. Aeration was carried out by drawing air through the culture by means of glass tubing equipped with a sintered-glass filter to break the air flow into fine bubbles. Radioactive substrates were added to the growth medium at the time of inoculation. At the end of 30 hr, the cells were collected by centrifugation at 32,000 x g for 10 min (all centrifugations performed in a Servall centrifuge, model RC2, at 0 C), washed once with distilled water, and resuspended in 5 volumes of ice-cold water. This suspension was mixed into 45 volumes of acetone at -10 C and allowed to stand overnight. The precipitate was collected by centrifugation at 32,000 x g for 10 min, and resuspended in 10 volumes of absolute acetone at -10 C. The residue was collected by centrifugation, dried in air at 4 C, and then treated with 15 volumes of ice-cold 7% perchloric acid by grinding in a mortar for 20 min at 4 C. The insoluble material was recovered by filtration through a layer of Hyflo Super-Cel on Whatman no. 5 filter paper in a Büchner funnel, washed two times each with 2 volumes of water, 95% alcohol, and absolute alcohol, and finally dried with a small amount of ether. The nucleic acids were extracted from the dried filter pad with 10% NaCl (see Bernstein and Foster, 1957), and the ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were separated (Bernstein, 1956). Ribonucleotides from RNA obtained by this procedure were converted to ribonucleosides by treatment with an acid prostatic phosphatase (Schmidt, Cubiles, and Thanhauser, 1947), and the nucleosides were separated on a column of Dowex 1 (HCOO-') and by paper chromatography (Bernstein and Sweet, 1958). For determination of the distribution of C^4 in the ribose, the C^4-nucleosides were diluted with unlabeled carrier nucleosides and fermented by *Lactobacillus plantarum* to acetate and lactate which then were further chemically degraded to yield each carbon separately as CO_2 (Bernstein, 19538). DNA was degraded to nucleosides by treatment with deoxyribonuclease and an aqueous extract of lyophilized venom from *Crotalus adamantis* (Bernstein and Sweet, 1938). The resulting deoxyribonucleosides were separated and purified by column chromatography and paper chromatography (Bernstein and Sweet, 1958). The C^4-thymidine obtained was diluted with unlabeled carrier thymidine and fermented with *Escherichia coli* ATCC 9723 to ethanol, CO_2, and acetate. The acetate and ethanol were further chemically degraded to obtain each carbon separately as CO_2 (Bernstein, Fossitt, and Sweet, 1958).

The specific activity of the ribose was determined by preparation (Löw, 1950) and combustion of furoic acid by the method of Van Slyke and Folch (1940). The specific activity of the deoxyribose was determined by preparation of levulinic acid and its combustion to CO_2 (Bernstein et al., 1958). Specific activities of the derivatives were determined as a check on the respective degradation procedures. In general, the activities found by total combustion of the respective derivatives were within ±5% of the values calculated from the sum of the activities of the individual carbons determined by the bacterial fermentation procedures; duplicate fermentations checked within 10% of each other.

Radioactive CO_2 was counted in a gas-phase proportional counter (Bernstein and Ballentine, 1950) to a standard error of 5% or less.

C^4-labeled substrates were obtained from Nuclear-Chicago Corp., DesPlaines, III.; unlabeled nucleosides were obtained from Sigma Chemical Co., St. Louis, Mo.

Results and Discussion

Biosynthesis of ribose. The patterns of distribution of isotope were determined in ribose isolated from cultures of *P. saccharophila* grown on specifically labeled C^4-glucose and gluconate substrates. These isotopic patterns were then compared with the theoretical patterns one would expect by known pathways of ribose synthesis to determine which pathways would account for the biosynthesis of ribose in this bacterium.

Formation of ribose from glucose via the oxidative decarboxylation of 6-phosphogluconate. By this pathway, carbons 2 through 6 of glucose furnish carbons 1 through 5 of ribose, respectively, while carbon 1 of glucose is lost as CO_2 (Horecker, Smyrniotis, and Seegmiller, 1951). Glucose-1-C^4 would not label ribose, whereas glucose-2-C^4 would label the pentose in carbon 1. Results of such experiments are shown in Table 1. The radioactivity of glucose-1-C^4 was not lost as CO_2 but
was retained and labeled carbon 1 of ribose with a relative specific activity (RSA) of 50 (experiments 1a and 1b). Glucose-2-C\textsuperscript{14} did not label carbon 1 of ribose to any significant extent but did label carbon 2 of ribose with a RSA of 51 (experiment 2). These results are not consistent with the formation of ribose via decarboxylation of 6-phosphogluconate and demonstrate that this pathway is not of major importance in pentose synthesis in P. saccharophila.

Formation of ribose via reactions catalyzed by transaldolase and transketolase. As shown in Fig. 1, in P. saccharophila carbons 1, 2, and 3 of glyceraldehyde-3-phosphate, a substrate for transketolase, would be derived from carbons 4, 5, and 6, respectively, of glucose via 2-keto-3-deoxy-6-phosphogluconate (Entner and Doudoroff, 1952). Carbons 1, 2, and 3 of glucose would not be converted to glyceraldehyde-3-phosphate by the normal glycolytic reactions, since fructose-1,6-diphosphate aldolase is missing in P. saccharophila (Entner and Doudoroff, 1952). A second substrate for transketolase, fructose-6-phosphate, is formed in P. saccharophila from glucose via glucose-6-phosphate (Fig. 1). Both the hexokinase and isomerase needed for this conversion have been found in P. saccharophila (Doudoroff et al., 1956). By the reactions shown in Fig. 1, two molecules of fructose-6-phosphate and one molecule of glyceraldehyde-3-phosphate are converted to two molecules of xylulose-5-phosphate and one molecule of ribose-5-phosphate. It is assumed in these experiments that ribose-5-phosphate is in equilibrium with xylulose-5-phosphate via D-xylulose-5-phosphate-3-epimerase (Ashwell and Hickman, 1957) and D-ribose-5-phosphate isomerase (Horecker, Smyrnios, and Klenow, 1953) and that all three pentose phosphates contribute to the synthesis of ribose of the nucleic acid. By the pathway shown in Fig. 1, glucose-1-C\textsuperscript{14} would label carbon 1 of ribose, glucose-2-C\textsuperscript{14} would label carbon 2 of ribose, and glucose-6-C\textsuperscript{14} would label carbon 5 of ribose with metabolically derived fructose-6-phosphate and triosephosphate as substrates for transaldolase and transketolase. As shown in Table 1, the isotopic patterns were generally in agreement with the predicted patterns. Glucose-1-C\textsuperscript{14} labeled carbon 1 of ribose with a RSA of 50 (experiments 1a and 1b), whereas the remaining four carbons of the pentose were almost unlabeled. Glucose-2-C\textsuperscript{14} labeled carbon 2 of ribose with a RSA of 51 (experiment 2), with the remaining four carbons of ribose again being unlabeled. Glucose-6-C\textsuperscript{14} labeled ribose pre-
dominantly in carbon 5 (RSA of 70 and 88 in experiments 3a and 3b, respectively). Since glucose-1-C\(^4\), glucose-2-C\(^4\), and glucose-6-C\(^4\) labeled ribose in carbons 1, 2, and 5, respectively, and this was the labeling pattern which would result from the series of reactions shown in Fig. 1, the major route of biosynthesis of ribose in \(P. \text{saccarophila}\) is assumed to be via transaldolase and transketolase. The proposed pathway does not explain how glucose-6-C\(^4\) would label carbon 1 of ribose as found in experiments 3a and 3b.

Similar experiments were performed with gluconate-1-C\(^4\) and gluconate-6-C\(^4\) as growth substrates. As shown in Table 1, when gluconate-1-C\(^4\) was used as a substrate (experiment 5), ribose was not labeled to a significant extent. The loss of radioactive carbon 1 of gluconate-1-C\(^4\) indicated that the synthesis of ribose could be from carbons 2 through 5 of gluconate in a reaction similar to the decarboxylation of 6-phosphogluconate, yielding ribulose 5-phosphate in which carbon 1 is lost as CO\(_2\). To test this hypothesis, gluconate-6-C\(^4\) was used as a carbon source. If the synthesis of ribose occurred via decarboxylation of 6-phosphogluconate, then gluconate-6-C\(^4\) would label carbon 5 of the pentose. Carbons 1 through 4 of ribose would not be labeled. As shown in experiment 6 (Table 1) gluconate-6-C\(^4\) labeled both carbons 1 and 5 of ribose with RSA values of 63 and 69, respectively. The labeling of carbons 1 and 5 similarly is not consistent with the formation of ribose via the decarboxylation of 6-phosphogluconate and indicates the possibility that a different pathway for the synthesis of ribose is present in gluconate-grown cells.

A second pathway for the synthesis of ribose which was considered for gluconate-grown cells was via transketolase and transaldolase. By the reactions shown in Fig. 1, both glyceraldehyde-3-phosphate and fructose-6-phosphate are required as substrates for transketolase. Glyceraldehyde-3-phosphate is formed from carbons 4, 5, and 6 of gluconate (Entner and Doudoroff, 1952) and would be available as a substrate in this system. However, the conversion of gluconate to fructose-6-phosphate has not been reported in \(P. \text{saccarophila}\). Therefore, it is necessary that either (i) fructose-6-phosphate, the required substrate for transketolase and transaldolase as shown in Fig. 1, is synthesized by an unknown route from gluconate and utilized for the synthesis of ribose, or (ii) a new pathway for the biosynthesis of ribose is present in \(P. \text{saccarophila}\) grown on gluconate which involves a different series of reactions than those shown in Fig. 1.

Effect of atmospheric CO\(_2\) on isotopic patterns. Glucose-U-C\(^4\) was used as a growth substrate to determine the extent of dilution of isotope in each carbon of ribose and deoxyribose by atmospheric CO\(_2\). Carbons of the pentoses which were not diluted by CO\(_2\) would have a RSA value of 100. As shown in Table 1 (experiment 4), carbons 2, 4, and 5 of ribose and carbon 1 of deoxyribose had approximately a 15% dilution of RSA by unlabeled atmospheric CO\(_2\), the remaining carbons of ribose and deoxyribose having RSA values near 100. When the RSA values of the carbons of ribose and deoxyribose in experiments 1 to 3b are corrected for these small dilutions, the absolute RSA values of particular carbons (carbons 2, 4, and 5 of ribose and carbon 1 of deoxyribose in experiments 1 to 3b) are lowered somewhat, but the isotopic patterns, and the interpretations of these patterns, in general, remain approximately the same.

Biosynthesis of deoxyribose. The radioisotope tracer technique was used to study the biosynthesis of deoxyribose in \(P. \text{saccarophila}\). The DNA was separated from the RNA, and the deoxyribose moiety was degraded to determine the isotope distribution in the pentose. This isotope distribution was compared with the theoretical pattern of isotopic patterns which would result from known pathways to determine whether these pathways were operative in \(P. \text{saccarophila}\).

Reduction of ribonucleotides to the corresponding deoxyribonucleotides. One such pathway is the reduction of ribonucleotides to deoxyribonucleotides in which ribose and deoxyribose would have identical labeling patterns. Comparison of the isotopic patterns in ribose and deoxyribose from glucose-1-C\(^4\), glucose-2-C\(^4\), and glucose-6-C\(^4\) and from gluconate-1-C\(^4\) and gluconate-6-C\(^4\) shows great similarity in the patterns (Table 1). In each case, the carbon atom containing the highest radioactivity was the same carbon number in ribose and deoxyribose. This similarity suggests that the major pathway for the biosynthesis of deoxyribose could be by reduction of a ribonucleotide to the corresponding deoxyribonucleotide. It is also possible that deoxyribose and ribose are synthesized by a pathway involving similarly labeled precursors.
However, these results do not decide between these two possible pathways.

Possible alternate pathways. Differences were found in isotope distribution between ribose and deoxyribose in experiments 3a, 3b, and 6. In experiments 3a and 3b, glucose-6-C$^14$ labeled carbon 2 of deoxyribose, whereas carbon 2 of ribose had almost no radioactivity. In experiment 6, carbons 2 and 4 of deoxyribose were labeled (RSA of 14 and 15, respectively), whereas carbons 2 and 4 of ribose were not labeled. These results do not conform to the above proposed pathways of reduction of ribonucleotides to deoxyribonucleotides or synthesis of ribose and deoxyribose from similarly labeled precursors. The observed differences in isotopic patterns may indicate the presence of an alternate pathway for the biosynthesis of deoxyribose in P. saccharophila. It is also possible that these differences may be a function of compartmentation of precursor pools or other environmental influences (Wright, Sable, and Baily, 1961).

Ghosh and Bernstein (1963) showed that NaIC$^14$O$_2$ given to young adult rats results in drastically different patterns of C$^14$ in the ribose and deoxyribose of the nucleic acids isolated from the liver.

The condensation of acetaldehyde and glyceraldehyde-3-phosphate to form 2-deoxy-D-ribose-5-phosphate by a reaction similar to that shown by Racker (1952) in E. coli was considered as a possible alternate pathway to direct reduction for synthesis of deoxyribose. Oxidation of glucose-2-C$^14$ in P. saccharophila results in the formation of pyruvate-2-C$^14$ (Entner and Douderoff, 1952). If decarboxylation of pyruvate occurs, acetaldehyde-1-C$^14$ would be formed. Condensation of this product with glyceraldehyde-3-phosphate would yield 2-deoxy-D-ribose-5-phosphate-1-C$^14$. In experiment 2, glucose-2-C$^14$ did not label carbon 1 of deoxyribose, indicating that this sequence of reactions did not occur. Additional evidence against the utilization of this pathway for the biosynthesis of deoxyribose was obtained by adding pyruvate-2-C$^14$ (assumed precursor of acetaldehyde and deoxyribose) to cultures of P. saccharophila growing on glucose. The deoxyribose of the nucleic acids from such cultures contained no radioactivity, indicating that the pathway involving pyruvate as a precursor of deoxyribose was not operative. It is possible that acetaldehyde may be derived from a source other than pyruvate, such as threonine (Boxer and Shronk, 1958); however, no evidence is available on this point in these experiments. Additional investigations will be necessary to determine whether 2-deoxy-D-ribose-5-phosphate aldolase could account for the incorporation of radioactivity into carbon 2 of deoxyribose in experiments 3a and 3b and into carbons 2 and 4 in experiment 6, or whether an alternate pathway for deoxyribose exists in P. saccharophila.

Experiments with glucose-1-C$^14$ and glucose-6-C$^14$ clearly indicated that decarboxylation of 2-deoxy-6-phosphogluconic acid (Lanning and Cohen, 1954) could not be a mechanism for the synthesis of deoxyribose in this system.

Acknowledgments

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Literature Cited


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