METABOLISM OF RIBOSE-1-C\textsuperscript{14} BY CELL-FREE EXTRACTS OF YEAST*

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The fermentation of ribose-5-phosphate by cell-free extracts of yeast is characterized by the equimolar formation of CO\textsubscript{2}, ethyl alcohol, and inorganic phosphate (1). Dickens (1) and Sable (2) suggested from their studies of pentose phosphate metabolism by cell-free yeast preparations that fermentation proceeded in the following manner: (1) cleavage of ribose-5-phosphate to a 2-carbon fragment and triose phosphate and (2) metabolism of the latter via classical glycolysis to CO\textsubscript{2}, ethanol, and inorganic phosphate. The fate of the 2-carbon component was undetermined.

Recently another pathway of pentose phosphate metabolism has been elucidated in liver and in higher plants by Horecker and coworkers (3, 4). In this pathway, ribose-5-phosphate is converted via ribulose-5-phosphate and sedoheptulose-7-phosphate to fructose-6-phosphate. The fructose-6-phosphate would be further metabolized by the classical pathway. In this pathway carbon atoms 1 and 2 of the pentose are involved in CO\textsubscript{2} and ethanol formation.

To determine the pattern of pentose fermentation by yeast extracts, ribose-1-C\textsuperscript{14} was used. Unlabeled ethanol and CO\textsubscript{2} would suggest the pattern described by Dickens and Sable, since in their scheme the end-products are derived from non-isotopic carbon atoms 3, 4, and 5 of the pentose. However, the appearance of tracer in the end-products would favor the scheme involving sedoheptulose, since carbon atom 1 enters the fructose-6-phosphate, which in turn gives rise to the alcohol and CO\textsubscript{2}. The results of the fermentation of ribose-1-C\textsuperscript{14} with yeast extracts are the subject of the present paper.

EXPERIMENTAL

Materials—Adenosine triphosphate (ATP), triphosphopyridine nucleotide (TPN), ribose, and ribose-5-phosphate were commercial products. Ribose-1-C\textsuperscript{14} and glucose-1-C\textsuperscript{14} were supplied by Dr. H. S. Isbell of the National Bureau of Standards. Ribonic acid and ribonic acid-5-phosphate prepared from ribose and ribose-5-phosphate by the hypiodite pro-

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procedure of Moore and Link (5) were kindly supplied by Dr. L. M. Paege of this laboratory. The barium salts of the phosphate esters were converted into potassium salts by dissolving them in 1 n HCl, adding a slight excess of 0.57 M K₂SO₄, and removing the resulting BaSO₄ by centrifugation. The supernatant solution was neutralized with 1 n KOH and diluted to the desired substrate concentration.

Analytical Methods—Alcohol was determined enzymatically (6) with crystalline alcohol dehydrogenase. Since the alcohol increase was small in proportion to the relatively high alcohol content of the cell-free preparations, all determinations were carried out in triplicate. With each determination, a sample of known alcohol content was included. The error among the three determinations was approximately 5 per cent. Inorganic phosphate was determined by the method of Fiske and Subbarow (7). Pentose was determined by the method of Mejbaum (8), modified by increasing the heating time to 40 minutes. Hexose monophosphate was determined by its ability to react with TPN in the presence of glucose-6-phosphate dehydrogenase and isomerase.

The CO₂ released during the fermentation of the ribose-1-C¹⁴ was collected and assayed as described previously (9). The ethanol was either converted to CO₂ by persulfate oxidation (10) or oxidized to acetic acid with 0.5 gm. of K₂Cr₂O₇ in 4 n H₂SO₄. The acetic acid was degraded by the method of Phares (11).

All C¹⁴ samples, in the form of barium carbonate, were assayed for activity with a methane flow beta proportional counter. Specific activity is expressed in this paper as millimicrocuries per mg. of carbon and total activity in millimicrocuries.

Enzyme Preparations—Pressed bottom beer yeast provided by the Schaefer Brewing Company, Brooklyn, was stirred with cold water in the cold room (4°) until the wash water was clear. Generally four washes were sufficient. After the yeast was sucked dry on a Büchner funnel, it was allowed to dry further at room temperature (20–25°). When the yeast was dry enough to crumble, it was passed through a No. 20 sieve. To prepare the yeast extract, 20 gm. of the dry yeast were incubated with 60 ml. of distilled water at 35–37° for 2.5 hours. The viscous solution was centrifuged in the cold room for 15 minutes at 18,000 × g. A light brown supernatant solution of approximately 25 ml., pH 6, was obtained. This solution was used for all experiments except one in which an attempt was made to determine intermediates formed during pentose metabolism. For the latter type of experiment, 5 ml. of yeast extract were dialyzed 16 hours against 3.5 liters of distilled water at 4°. A precipitate which formed after dialysis was removed by centrifugation and discarded.
Results

Fermentation of Ribose and Ribose-5-phosphate—Since C\(^{14}\)-ribose is more readily available than the labeled phosphorylated pentose, an attempt was made to determine whether our yeast extracts could ferment ribose as well as ribose-5-phosphate. That ribose is fermented rapidly after a slight lag period is evident in Fig. 1. Fig. 1 also indicates that the lag period is decreased and the rate of fermentation is increased by the addition of ATP, suggesting that the ribose is phosphorylated. Sable (2) has given evidence...
that brewers' yeast extracts possess a ribokinase and that the product of the phosphorylation is ribose-5-phosphate. Fermentation of ribose is contrary to the experience of Dickens (1) with extracts of Lowenbrau, Munich, bottom beer yeast. It is therefore of interest to note that of three types of brewers' yeast tested for their ability to attack ribose, only the Schaefer strain yielded extracts which were active in this respect.

The yield of CO₂ is similar with both substrates. In a typical experiment, 10 μmoles of ribose yielded 12.1 μmoles of CO₂, while 60 μmoles of ribose-5-phosphate gave rise to 56.2 μmoles of CO₂. This can be taken as further evidence of a common pathway of fermentation for ribose and ribose-5-phosphate.

### Table I

**Stoichiometry of Ribose-5-phosphate Fermentation**

<table>
<thead>
<tr>
<th>Pentose</th>
<th>CO₂</th>
<th>PO₄</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmoles</td>
<td>μmoles</td>
<td>μmoles</td>
<td>μmoles</td>
</tr>
<tr>
<td>Initial</td>
<td>133</td>
<td>435</td>
<td>480</td>
</tr>
<tr>
<td>Final</td>
<td>76</td>
<td>475</td>
<td>518</td>
</tr>
<tr>
<td>-57</td>
<td>+54</td>
<td>+40</td>
<td>+38</td>
</tr>
</tbody>
</table>

The Warburg vessel contained 5.0 ml. of yeast extract. The gas space contained helium; temperature 37.5°. The reaction was begun by tipping 75 μmoles of ribose-5-phosphate from one side arm and was stopped by the addition of 0.5 ml. of 50 percent trichloroacetic acid. The final CO₂ is corrected for CO₂ (21 μmoles) liberated by adding trichloroacetic acid to a test system in which ribose-phosphate was omitted. The precipitate was removed by centrifugation, and the supernatant solution was brought to pH 7.5 with 4 N KOH. Aliquots of this solution were analyzed for pentose, phosphate, and ethanol.

**Products of Ribose-5-phosphate Fermentation**—In agreement with Dickens (1), we found that ribose-5-phosphate was fermented by the yeast extracts to nearly equimolar amounts of inorganic phosphate, ethanol, and CO₂ (Table I). The molar amounts of ethanol and phosphate formed were approximately equal, but consistently slightly less than the CO₂.

**Intermediates in Pentose Phosphate Metabolism**—After dialysis, the preparations lost their ability to form ethanol and CO₂ from pentose phosphate. However, the extracts rapidly metabolized ribose-5-phosphate, with an initial rapid accumulation of hexose monophosphate (Fig. 2). After the initial phase, hexose monophosphate formation ceased. A similar situation has been found in liver extracts (3, 12).

**Isotope Distribution in Ethyl Alcohol and Carbon Dioxide Formed from Ribose-1-C¹⁴**—Both end-products, CO₂ and ethyl alcohol (methyl carbon), contained significant quantities of isotope (Table II). A comparison of the
Fig. 2. Hexose monophosphate formation from ribose-5-phosphate. The incubation mixture contained 15.0 µmoles of ribose-5-phosphate and 1.0 ml. of dialyzed yeast extract in a total volume of 1.1 ml. Incubation was at 34°. At the intervals indicated, aliquots were diluted with 5 volumes of water, heated for 2 minutes at 100°, and centrifuged to remove the coagulated protein.

TABLE II

Location of Tracer in Ethyl Alcohol and Carbon Dioxide Formed from Ribose-1-C¹⁴ by Yeast Extracts

The results are expressed in millimicromoles per mg. of carbon.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>CO₂</th>
<th>Ethyl alcohol</th>
<th>Total oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CH₃OH</td>
<td>CH₂</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>15.3</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>14.6</td>
<td>13.3</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>15.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

In Experiments 1, 2, and 3 the amounts of CO₂ obtained in the presence of ribose were 260, 352, and 270 µmoles, respectively; the amounts in the corresponding control runs were 88, 89, and 88 µmoles. The respective amounts of ethanol in the presence of ribose were 1648, 1950, and 1918 µmoles; the amounts in the corresponding controls were 1500, 1690, and 1792 µmoles. A different cell-free preparation was used in each experiment.

For each experiment, CO₂ and ethanol from two test systems were pooled. The third vessel contained the control, in which ribose was omitted. Each Warburg vessel contained 10.0 ml. of yeast extract and 50 µmoles of ATP. The reaction was begun by tipping in 150 µmoles of ribose-1-C¹⁴ (specific activity of 12.0). The total volume was 10.7 ml. The gas space contained helium; temperature 37.5°. After 4 hours, when CO₂ evolution had practically ceased, 2 ml. of 50 per cent trichloroacetic acid were tipped in. The CO₂ was collected by adding KOH through the venting plug (9). After complete removal of the CO₂, the vessel contents were centrifuged. The supernatant solution was distilled to remove the alcohol. The alcohol was oxidized to acetic acid with 0.5 gm. of K₂Cr₂O₇ in 4 N H₂SO₄ at 90° for 1.5 hours. The acetic acid, which was distilled from the reaction flask and titrated with 0.07 N KOH, was made to volume. Part was removed for total oxidation (10), and the remainder was evaporated on the steam bath to dryness and degraded (11). The specific activity values are corrected for endogenous dilution.
specific activity of the methyl carbon of ethanol with that of carbon atom 1 of pentose indicates an approximate 2-fold dilution, while a similar comparison of CO₂ with pentose shows a 4-fold dilution.

**DISCUSSION**

The transfer of the bulk of the isotope originally located in carbon atom 1 of ribose to the methyl carbon of alcohol and to CO₂ strongly suggests that the major pathway of pentose metabolism by the yeast extracts involves the following: (1) a conversion of pentose to hexose monophosphate via a transketolase-transaldolase sequence of reactions, (2) the conversion of the hexose monophosphate to CO₂ and ethanol via classical yeast glycolysis (13).

To account for the tracer pattern found in these extracts, it is apparent that more than one reaction must be considered. The scheme involving the sequences of reactions proposed for the conversion of pentose phosphate to hexose monophosphate by liver and pea root extracts (3, 4) can also be applied to our yeast extracts. However, to account for the quantitative differences between the labeling of the end-products obtained with the yeast and those obtained with liver and pea roots, the assumption must be made that the rates of the various sequences differ in the several extracts.

According to the transaldolase-transketolase sequence, 1-labeled pentose

\[
\begin{align*}
\text{C} & \xrightarrow{\text{transketolase}} \text{C} + \text{C} \\
\text{C} & \xrightarrow{\text{transaldolase}} \text{C} + \text{C}
\end{align*}
\]
(tracer indicated by *) would be converted to hexose monophosphate in the two ways illustrated in the accompanying diagram. This sequence of reactions would yield hexose monophosphate labeled in carbon atoms 1 and 3, the isotope in carbon atom 1 being equal to that of carbon atom 1 of the pentose phosphate, while carbon atom 3 would contain half as much. This product would give rise to CO₂ with 0.25 times the specific activity of pentose phosphate carbon atom 1, while the methyl carbon atom of ethanol would have 0.50 times the specific activity of carbon atom 1 of the pentose. Since the observed ratios are 0.25 and 0.4, respectively, our data fit this sequence.

Pentose phosphate converted to CO₂ and ethanol via a transaldolase-transketolase sequence should show a stoichiometry of 3 pentose phosphate → 2.5 hexose monophosphate → 5CO₂ + 5 ethanol + 3 inorganic phosphate. The stoichiometry shown in Table I as well as that reported by Dickens (1) does not fit this balance. Since the fermentation did not go to completion, it is not unlikely that appreciable quantities of intermediates accumulate.

Other possible patterns of ribose metabolism may be considered: (1) oxidation of ribose to ribonic acid or conversion of ribose to ribose-5-phosphate, followed by oxidation to ribonic acid-5-phosphate, then a cleavage of the acids or (2) a recycling of the hexose monophosphate via the direct oxidation pathway. The first pathway is eliminated, since our extracts did not attack ribonic acid or ribonic acid-5-phosphate. To test the second possibility, glucose-1-C¹⁴ was fermented by these extracts. If a considerable amount of the glucose was fermented by an anaerobic glucose-6-phosphate shunt, then a considerable amount of tracer should be located in the carbon dioxide; however, only 3 per cent was found.

**SUMMARY**

The conversion of ribose-1-C¹⁴ to ethyl alcohol and CO₂ by cell-free extracts of beer yeast has been investigated.

The extracts converted ribose-1-C¹⁴ (60)¹ into methyl-labeled alcohol (20 to 27)¹ and C¹⁴O₂ (15).¹ Under conditions in which CO₂ and alcohol production was inhibited, hexose monophosphate accumulated. From the isotope data it is concluded that the pentose is converted to hexose monophosphate by a transketolase-transaldolase sequence of reactions, followed by a conversion of the hexose monophosphate to the end-products via classical yeast glycolysis.

Metabolism of the pentose via ribonic acid, ribonic acid-5-phosphate, or a recycling of the hexose monophosphate via an anaerobic glucose-6-phosphate shunt did not appear to occur in these preparations.

¹ Millimicrocuries per mg. of carbon.
BIBLIOGRAPHY

METABOLISM OF RIBOSE-1-\textsuperscript{14} BY CELL-FREE EXTRACTS OF YEAST
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