The Metabolism of Hydroxypyruvate

II. THE ENZYMATIC OXIDATION AND DECARBOXYLATION OF HYDROXYPYRUVATE*

JERRY L. HEDRICK† AND H. J. SALLACH

From the Department of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin

(Received for publication, January 3, 1961)

Our knowledge of the metabolic role of hydroxypyruvate has expanded greatly in recent years. In animal systems, hydroxypyruvate has been shown to participate in the following reactions: (a) transamination with alanine to yield serine (2), (b) reduction to L-glyceric acid in the presence of DPNH and lactate dehydrogenase (3), (c) conversion to glucose (4), (d) decarboxylation and subsequent condensation with glyceraldehyde to yield xylulose (5), and (e) oxidation by purified preparations of pyruvic oxidase (6). However, the metabolic origin and final catabolic fate of hydroxypyruvate remain unknown. As has been previously reported (7), the compound is rapidly metabolized by rat liver homogenates. In an effort to elucidate the catabolic reactions of hydroxypyruvate, it was necessary to determine to what extent it is metabolized by the above mentioned reactions. The purpose of the studies presented here is an evaluation of the participation of hydroxypyruvate in some of these reactions with a washed residue preparation from rat liver.

EXPERIMENTAL PROCEDURE

Materials—The lithium salt of hydroxypyruvate was prepared according to the method of Dickens and Williamson (8). Radioactive hydroxypyruvate was synthesized as described in a preceding paper (9). Hydroxypyruvate was assayed spectrophotometrically with DPNH and hydroxypyruvate reductase from parsley (10). Pyruvic acid used as a substrate, and in the preparation of bromopyruvate, was redistilled three times before use (11). ATP, DPN, and other cofactors and compounds were commercial preparations.

Enzyme Preparation—The enzyme source was a washed residue preparation from rat liver prepared essentially by the method of Lehninger and Kennedy (12). Livers from rats weighing from 175 to 200 g were placed in a Potter-Elvehjem homogenizer and homogenized with 2 volumes of 0.13 M sodium phosphate buffer, pH 6.8. The total volume was 3.0 ml. After a 5-minute equilibration period, the substrate was tipped into the center well and diluted to 10 ml. A U40Z analysis was carried out as previously described (9). Other experimental details, when differing from those above, are presented with the individual experiments.

Deficient Rats—Vitamin-deficient rats were obtained by feeding rats a synthetic diet devoid of thiamine or riboflavin but complete in all other respects. Control animals were fed the same synthetic diet except that thiamine and riboflavin were added. Weanling rats weighing an average of 50 g were used. After 26 days of feeding, the thiamine-deficient rats were killed. They weighed an average of 53 g compared to 153 g for the control animals. The riboflavin-deficient rats were killed after 53 days of feeding. They weighed an average of 73 g compared to 189 g for the control group.

RESULTS

All values reported have been corrected for endogenous respiration. In addition, they have also been corrected for the nonenzymatic decarboxylation and autoxidation observed with a hydroxypyruvate control. As previously described, hydroxypyruvate is a labile compound even under the mild conditions used in enzymatic experiments (9).

The effect of DPN, ATP, and arsenite upon the oxygen uptake and decarboxylation of pyruvate and hydroxypyruvate in this system is presented in Table I. The addition of DPN was required for the oxidation of pyruvate, but not for either the oxidation or decarboxylation of hydroxypyruvate. Similar results were obtained with TPN. As would be expected in this system, added ATP was not required for the oxidation of pyruvate nor did it influence the oxidation or decarboxylation of hydroxypyruvate. Experiments with Tris buffer and various metal ions to form complexes of phosphate did not alter hydroxypyruvate oxidation or decarboxylation. Furthermore, phosphohydroxypyruvate was not oxidized in this system.

Arsenate is known to be an inhibitor of the pyruvic dehydrogenase system (13) and was therefore investigated in these
The incubation mixture contained: 50 μmoles of pyruvate or 45.5 μmoles of 1-C14-hydroxypyruvate; phosphate buffer, pH 7.0; Mg++, 1.2 X 10^-2 M; arsenite, 8 X 10^-2 M; ATP, 1 X 10^-3 M; DPN, 1 X 10^-3 M.

<table>
<thead>
<tr>
<th>Reaction system</th>
<th>O2 uptake</th>
<th>CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>37</td>
<td>μl</td>
</tr>
<tr>
<td>Hydroxypyruvate</td>
<td>203</td>
<td>4.5</td>
</tr>
<tr>
<td>Pyruvate + DPN</td>
<td>372</td>
<td>11</td>
</tr>
<tr>
<td>Hydroxypyruvate + DPN</td>
<td>199</td>
<td>4.1</td>
</tr>
<tr>
<td>Pyruvate + arsenite + DPN</td>
<td>1-1</td>
<td>3.1</td>
</tr>
<tr>
<td>Hydroxypyruvate + arsenite + DPN</td>
<td>209</td>
<td>3.1</td>
</tr>
<tr>
<td>Pyruvate + ATP</td>
<td>20</td>
<td>3.9</td>
</tr>
<tr>
<td>Hydroxypyruvate + ATP</td>
<td>200</td>
<td>3.9</td>
</tr>
<tr>
<td>Pyruvate + DPN + ATP</td>
<td>372</td>
<td>3.6</td>
</tr>
<tr>
<td>Hydroxypyruvate + DPN + ATP</td>
<td>213</td>
<td>3.6</td>
</tr>
</tbody>
</table>

The oxidation of hydroxypyruvate, however, was decreased 25 to 30% in the presence of arsenite. This may be a measure of the extent to which hydroxypyruvate enters into the pyruvic dehydrogenase series of reactions.

In order to obtain an insight into what cofactors, if any, may be involved in hydroxypyruvate oxidation and decarboxylation in this system, enzyme preparations from thiamine- and riboflavin-deficient rats were investigated. The oxidation of hydroxypyruvate, in the case of riboflavin deficiency (Table II), was lowered some 75% in comparison to that observed with a control animal. Addition of flavin mononucleotide or FAD did not restore oxygen uptake to the normal level. In contrast, the decarboxylation of hydroxypyruvate observed was about the same in preparations from either the riboflavin-deficient or normal control animals. It appears that a flavin may be involved in the oxidation of hydroxypyruvate.

Pyruvate oxidation, on the other hand, was markedly stimulated in the riboflavin-deficient preparations. Oxygen uptake was 3 times greater in the deficient preparations than it was in that from the normal animal in the presence of DPN. The figures represent actual participation in the pyruvic dehydrogenase system rather than some other reaction because there was a release of CO2 paralleling the increased oxygen uptake. Flavin has not been indicated to be involved directly in the animal pyruvic dehydrogenase system. The reason for this large increase in oxygen uptake in the case of pyruvate is unknown.

Table III indicates the effect of thiamine on hydroxypyruvate oxidation and decarboxylation and on pyruvate oxidation. The oxidation of hydroxypyruvate observed was the same in normal and in thiamine-deficient preparations. Thiamine deficiency resulted in a slight decrease in the decarboxylation of hydroxypyruvate. Because pyruvate oxidation was reduced to a very low level in these preparations, the small decrease in the decarboxylation of hydroxypyruvate observed may represent the extent to which this compound is decarboxylated by the pyruvic dehydrogenase system or some other thiamine-requiring reaction. Thiamine, however, does not appear to be involved in the main reactions resulting in either the decarboxylation or oxidation of hydroxypyruvate.

1, 2, or 3-C14-labeled hydroxypyruvates were used to determine to what extent the hydroxypyruvate molecule was degraded to CO2. The results (Table IV) are expressed as the percentage of C-1 of hydroxypyruvate as C1402 released. It can be seen that C-1 is the major carbon lost. Further degradation of the decarboxylation product(s) occurs only to a limited extent in this system. Very little C1402 originating from C-3 of hydroxypyruvate was observed.

**DISCUSSION**

Hydroxypyruvate has been found to undergo a wide variety of reactions in plants and animals. A fairly complete listing of
TABLE IV
C^14O_2 production from C^{14}labeled hydroxypyruvates
The incubation mixture contained: 50 pmol of hydroxypyruvate and phosphate buffer, pH 6.8.

<table>
<thead>
<tr>
<th></th>
<th>CO_2, % C^{14} released</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxypyruvate-1-C^{14}</td>
<td>(100)</td>
</tr>
<tr>
<td>Hydroxypyruvate-2-C^{14}</td>
<td>13.8</td>
</tr>
<tr>
<td>Hydroxypyruvate-3-C^{14}</td>
<td>3.1</td>
</tr>
</tbody>
</table>

These reactions can be found in papers by Fukunaga (5) and Dickens and Williamson (4). One of the more important reactions in which hydroxypyruvate could participate in a washed residue preparation would be that of pyruvic dehydrogenase. In order to evaluate this pathway of hydroxypyruvate oxidation, the metabolism of hydroxypyruvate and pyruvate were compared with a washed residue preparation, in response to various cofactors and inhibitors.

Pyruvate oxidation requires the addition of DPN in this system and is completely inhibited by 8 \times 10^{-3} M arsenite. In contrast, neither of these two compounds has any effect upon the oxidation or decarboxylation of hydroxypyruvate. The metabolism of pyruvate and hydroxypyruvate responds differently to riboflavin deficiency. Hydroxypyruvate oxidation was decreased and decarboxylation increased slightly. On the other hand, pyruvate oxidation was greatly increased. Thiamine is known to be an important cofactor in pyruvate oxidation. As would be expected, thiamine deficiency greatly reduced the pyruvate oxidation but did not significantly alter either the oxidation or decarboxylation of hydroxypyruvate from that observed with the normal controls.

Thus, it seems clear that although hydroxypyruvate can participate in a purified pyruvic oxidase system as shown by Jagannathan and Schweet (6), it does not do so to any great extent in this system. A measure of the extent to which hydroxypyruvate is decarboxylated by pyruvic oxidase may be the decrease in decarboxylation when the system is inhibited by arsenite. This decrease is paralleled by a decrease in decarboxylation when a thiamine-deficient preparation is used.

The lack of response of hydroxypyruvate metabolism to thiamine deficiency tends to minimize participation in reactions requiring thiamine. Thiamine is of course an important component of the transketolase system as well as pyruvic dehydrogenase. If hydroxypyruvate were primarily participating in such reactions, one should observe a drastic lowering of its decarboxylation in the thiamine-deficient preparation. No such reaction was detected. The enzymes characteristic of the pentose cycle have been shown to be extramitochondrial enzymes (14). Therefore, the amount of transketolase present in a washed residue preparation should be small.

Apparently, hydroxypyruvate is undergoing at least two reactions in this system which have not been fully described before for an animal system: that of nonoxidative decarboxylation and oxidation.

The decarboxylation shows no response to added DPN, TPN, or cytochrome c. Hydroxypyruvate decarboxylation was unaffected by anaerobic conditions. The deficiency experiments indicate that a flavin component does not appear to be involved in the decarboxylation. C-1 of hydroxypyruvate seems to be the major carbon lost as CO_2. Neither inorganic phosphate nor the phosphorylated derivative (phosphohydroxypyruvate) appear to be involved in the oxidation of hydroxypyruvate.

Kuratomi and Fukunaga (15) have described in a preliminary note a Mg^{2+}-requiring system from rat liver which apparently can decarboxylate hydroxypyruvate. Added thiamine pyrophosphate had no effect upon the decarboxylation of hydroxypyruvate. These workers identified the decarboxylation product as glycolaldehyde. Their system may or may not be the same as the one studied here, but further purification and characterization of these systems must be achieved before this can be determined.

The oxidation of hydroxypyruvate may involve a flavin component. Thus far, this is the only compound found to have any effect upon the oxygen uptake. The oxygen uptake in a preparation from a riboflavin-deficient rat could not be restored by the addition of flavin mononucleotide or FAD. This finding is similar to observations by Burch et al. (16) that the oxidation of succinate, \( \beta \)-hydroxybutyrate, or \( \alpha \)-ketoglutarate by mitochondria from riboflavin-deficient rats could not be restored to normal by the addition of FAD or flavin mononucleotide. However, the increase in oxygen uptake when pyruvate and DPN are used as substrates is in direct contrast to their findings for \( \alpha \)-ketoglutarate and \( \beta \)-hydroxybutyrate. The explanation for this discrepancy is unknown.

The oxidation and decarboxylation steps seem to be two separate divergent reactions rather than sequential reactions. Since decarboxylation is nonoxidative, glycolaldehyde is a possibility as the decarboxylation product. If the reactions were sequential, glycolaldehyde should be further oxidized. However, when glycolaldehyde is added as a substrate, it is oxidized to a very small extent. The oxygen uptake is much too high in relation to CO_2 release for decarboxylation to precede oxidation. Oxidation cannot precede decarboxylation as anaerobic conditions have no effect upon decarboxylation. Results to be published subsequently on the effect of metal ions in these two reactions also indicate that they are two separate divergent reactions.

The work described here is only intended to be indicative of approaches for future research on the metabolism of hydroxypyruvate. Further purification of these enzymes and characterization of the enzymatic oxidation and decarboxylation of hydroxypyruvate are in progress.

**REFERENCES**

2. **Sallach, H. J., J. Biol. Chem., 223, 1101 (1956).**
3. **Meister, A., J. Biol. Chem., 184, 117 (1950).**
The Metabolism of Hydroxypyruvate: II. THE ENZYMATIC OXIDATION AND DECARBOXYLATION OF HYDROXYPYRUvATE
Jerry L. Hedrick and H. J. Sallach


Access the most updated version of this article at http://www.jbc.org/content/236/7/1872.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/236/7/1872.citation.full.html#ref-list-1