CONVERSION OF ACETATE AND PYRUVATE TO GLUTAMIC ACID IN YEAST*

BY CHIH H. WANG, BERT E. CHRISTENSEN, AND VERNON H. CHELDELIN

(From the Department of Chemistry, Oregon State College, Corvallis, Oregon)

(Received for publication, July 24, 1952)

Studies in this laboratory have aimed at a comparison of pyruvate and acetate as carbon sources for amino acids in bakers' yeast (1–3). Aspartic acid in particular was shown (3) to arise from pyruvate largely through a C₅-C₁ condensation, and from acetate perhaps via a direct coupling of C₂ units.

In the present paper, these studies have been extended to include the formation of glutamic acid. With isotopic acetate and pyruvate as substrates, the yield of glutamic acid and the intramolecular distribution of C¹⁴ suggest that this amino acid is formed from C₄ acids via the Krebs cycle in this organism. Assuming that citrate is formed as an intermediate, the results further confirm Ogston's principle (4–5) of the unsymmetrical nature of the latter compound.

EXPERIMENTAL

The organism used was Fleischmann's bakers' yeast. Three samples were employed: two were cultured on CH₃C¹⁴OOCOOH, one in oxygen and the other in nitrogen, and the third on CH₃C¹⁴OOH in oxygen. The details of these fermentations have been presented previously (1).

Glutamic acid was isolated from the protein hydrolysates of these yeast fractions (2) in the form of its hydrochloride, and its purity established by paper chromatography. It was then diluted with non-isotopic glutamic acid hydrochloride and subjected to the following degradation processes, as outlined in the diagram. (a) Combustion provided a measure of the total activity. (b) Ninhydrin decarboxylation (6) removed C₁. (c) Schmidt's reaction (7, 8) yielded α,γ-diaminobutyric acid, which was isolated as the picrate. Combustion of the latter gave the sum of the activity of carbons 1 to 4 directly and C₅ by difference. (d) Silver oxide oxidation (9) of α,γ-diaminobutyric acid yielded β-alanine, which on combustion gave the activity of C₂+3+4. (e) KOH-NaOH fusion of β-alanine (10–12) led to the formation of acetic acid. The latter was isolated as its barium salt, which on combustion gave the activity of C₂+3 directly and C₄ by

* This research was supported by contract No. AT(45-1)-301 from the Atomic Energy Commission. Published with the approval of the Monographs Publications Committee, Research paper No. 211, School of Science, Department of Chemistry.
difference. A portion of the barium acetate formed was then pyrolyzed to acetone and then converted to iodoform (13). Combustion of iodoform gave the activity of C₂ directly and C₃ by difference.

In the silver oxide oxidation, 10 mM of α,γ-diaminobutyric acid dihydrochloride were dissolved in 100 ml. of water and stirred with an equivalent amount of freshly prepared silver oxide for 30 minutes. The filtered solution of α,γ-diaminobutyric acid was then mixed with 60 mM of freshly prepared silver oxide suspension in 50 ml. of water. The mixture was refluxed for 12 hours on a heating mantle. On cooling, the mixture was filtered and the silver residue washed thoroughly with water. The combined filtrate was then evaporated under reduced pressure to about 10 ml. and cleared of silver ion with hydrogen sulfide. The mixture was again filtered and the filtrate evaporated under reduced pressure to a syrup. 10 ml. of 70 per cent ethanol were added, followed by a solution of 30 mM of 2-nitro-1,3-indandione (14) in 50 ml. of hot ethanol. After standing in the refrigerator for 36 hours, the separated crystals were collected and recrystallized from 80 per cent ethanol. The melting point of β-alanine-2-nitro-1,3-indandionate was 192–195° with decomposition (corrected). Yield, 40 to 45 per cent based on α,γ-diaminobutyric acid.
The 2-nitro-1,3-indandionate was converted to β-alanine by the addition of hydrochloric acid, followed by ether extraction. The extracted aqueous solution of β-alanine hydrochloride was evaporated under reduced pressure to dryness to remove free HCl and the residue was dissolved in a few ml. of water. Freshly prepared silver oxide (10 per cent excess of the equivalent quantity of β-alanine) was used to remove all of the chloride ion and the mixture then filtered. The filtrate was saturated with hydrogen sulfide, filtered, and evaporated to a small volume under reduced pressure. On further evaporation in a vacuum desiccator in the presence of P₂O₅, β-alanine was obtained as colorless crystals. Yield, 38 to 42 per cent based on diaminobutyric acid; m.p. 196° with decomposition (corrected).

KOH-NaOH fusion of β-alanine was effected according to the procedure given by Baddiley et al. (12) for the oxidation of tyrosine to p-hydroxybenzoic acid. 4 mM of β-alanine were added in portions to a mixture of 50 mM of KOH and 70 mM of NaOH in a silver crucible preheated to 340°. In about 30 minutes the ammonia evolution ceased and the fusion was then complete. On cooling, the mixture was dissolved in 50 ml. of water and neutralized carefully with dilute sulfuric acid in an ice bath. The solution was then transferred to a 3-necked flask equipped with a reflux condenser, a dropping funnel, and a gas disperser. Sulfuric acid was added to a concentration of 3 N, after which 10 ml. of 0.5 N KMnO₄ were added to remove side reaction products from the fusion process. The mixture was refluxed for about 1 hour, and a slight excess of saturated sodium oxalate solution was introduced to remove the residual permanganate. The solution was then titrated with dilute permanganate to remove excess oxalate and flushed with CO₂-free nitrogen gas for a few minutes at the boiling point. The steam-distilled acetic acid was recovered from the distillate as the barium salt. Part of the barium acetate was burned for activity determination and the rest used in the pyrolysis reaction for the conversion to acetone and iodoform.

Radioactivities were determined as barium carbonate in the conventional manner; counting data were corrected for background and self-absorption.

RESULTS AND DISCUSSION

The intramolecular distribution of C¹⁴ in the glutamic acid formed by the acetate and pyruvate yeasts is given in Table I. 4 gm. of yeast were inoculated into each medium at the start of the experiments.

The conversion of acetate to glutamic acid resulted in isotopic labeling exclusively in the carboxyl groups, the ratio of activities in the γ-α-carboxyl approaching 2:1. Such distribution is precisely in accord with expectations if the citric acid cycle is assumed to operate extensively, and, if
the oxalacetate were unlabeled in the central carbons, as found (3), the above distribution would obtain after one complete turn of the cycle. As a corollary, the original isotopic concentration in the oxalacetate carboxyl groups will have no effect upon the final distribution in \(\alpha\)-ketoglutarate or glutamic acid unless the cyclic activity is reduced to less than one complete turn. The fact that virtually no growth occurred in the acetate yeast sample (1) may account for the extensive cycling observed here.

The distribution of activity in the glutamic acid isolated from pyruvate yeast was markedly different from that described for acetate. In the

**TABLE I**

*Distribution of \(^{14}C\) in Glutamic Acid from Yeast Utilizing CH\(_3\)C\(^{14}OH\) or CH\(_3\)C\(^{14}O\)COOH*

<table>
<thead>
<tr>
<th>Carbon atom</th>
<th>Aerobic acetate</th>
<th>Aerobic pyruvate</th>
<th>Anaerobic pyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c.p.m. (\times 10^6)</td>
<td>per cent of total</td>
<td>c.p.m. (\times 10^6)</td>
</tr>
<tr>
<td>Total</td>
<td>4.17</td>
<td>100</td>
<td>14.4</td>
</tr>
<tr>
<td>1, COOH</td>
<td>1.42</td>
<td>34</td>
<td>3.67</td>
</tr>
<tr>
<td>2, CHNH(_2)</td>
<td>0</td>
<td>0</td>
<td>2.48</td>
</tr>
<tr>
<td>3, CH(_2)</td>
<td>0</td>
<td>0</td>
<td>2.77</td>
</tr>
<tr>
<td>4, (\gamma)</td>
<td>0</td>
<td>0</td>
<td>-0.03†</td>
</tr>
<tr>
<td>5, COOH</td>
<td>2.75</td>
<td>66</td>
<td>5.57</td>
</tr>
<tr>
<td>Ratio (\gamma)-COOH/(\alpha)-COOH</td>
<td>1.94</td>
<td>1.55</td>
<td>1.85</td>
</tr>
</tbody>
</table>

* Specific activity (total) is expressed as counts per minute per millimole of glutamic acid; the activities of individual carbon atoms are counts per minute per millimole of carbon.
† This value is insignificant as indicated by statistical analysis.

aerobic sample, over one-third of the total activity in the molecule was present in carbon atoms 2 and 3; moreover, the isotope was equally distributed between these two atoms.\(^1\) No activity was found in carbon 4 of either the aerobic or anaerobic sample.\(^1\) The \(\gamma\)-\(\alpha\)-carboxyl activity ratio was appreciably below 2:1, especially in the aerobic sample. Finally, it should be recalled that extremely large amounts of this amino acid were formed (2), perhaps one-half or more of the total growth of the yeast being represented by this compound.

With appropriate restrictions, operation of the citric acid cycle also appears adequate to explain the formation of glutamic acid from pyruvate in these experiments. The total absence of isotope in carbon 4 is in agree-

\(^1\) According to statistical treatment of the analytical data.
ment with this type of condensation, as is the greater activity of the \(\gamma\)-over the \(\alpha\)-carboxyl. On the other hand, the low ratio of 1.55:1 for activity of these groups indicates that much of the glutamic acid formed must have arisen with much less cyclic activity, and thus the additional activity introduced into the \(\beta\)-carboxyl of oxalacetate through \(C_2 + C_1\) condensation (3) could be reflected in the \(\alpha\)-carboxyl of glutamic acid. The very high yield of glutamic acid agrees with this concept, as does also the high specific activity (14 \(\times\) \(10^5\) c.p.m. per mm compared to 18.7 \(\times\) \(10^5\) in the original pyruvic acid (1)). The presence of isotope in high concentration in carbons 2 and 3 is also to be expected if, as shown previously (3), a \(C_3-C_1\) condensation operates in yeast to provide \(C_4\) acids from pyruvate, and if these centrally labeled acids condense with the active \(C_2\) unit that is formed from the administered carbonyl-labeled pyruvate.

The equal labeling of carbons 2 and 3 in glutamic acid must be explained (in terms of the Krebs cycle) by the availability of a symmetrically labeled \(C_4\) acid as a condensing partner. However, it will be recalled that the aspartic acid from this yeast was not so symmetrically labeled (only 11 to 28 per cent of the aspartic acid molecules became randomized (3)). Two explanations appear possible. (1) The \(C_4\) acid (oxalacetate) that condenses via the cycle is in equilibrium with a symmetrical \(C_4\) acid (e.g., fumarate) and therefore represents a different species of oxalacetate from that which produces aspartic acid. This may not be unreasonable if it is assumed that the intermediate enzymic reactions leading to glutamate formation occur without release of their substrate molecules. If this were true, no oxalacetate “pool” would be expected to exist, at least with respect to the oxalacetate-aspartic acid system. (2) Alternatively, it is possible that the main condensation occurs in this system between a \(C_2\) unit and a symmetrical \(C_4\) acid such as fumarate. Such a condensation would necessarily be oxidative in type. Traditionally, the first explanation would appear to be favored, since the second one is not given support by other published information. A final analysis of these possibilities will have to await studies with cell-free systems.

The radioactivity of the glutamic acid from the anaerobic pyruvate sample recalls the experiments with aspartic acid (3); in both, the distribution of isotope is intermediate in pattern between those of the acetate and aerobic pyruvate yeasts. The lower specific activity of carbons 2 and 3 in glutamic acid from anaerobic pyruvate indicates lowered carboxylating activity in the formation of \(C_4\) acids, a reaffirmation of the previous observations on aspartic acid formation (3).

Although the present experiments were not designed to bear upon the question of the symmetry of the citric acid molecule, the strongly preferential labeling of the \(\gamma\)-carboxyl group in glutamic acid appears to furnish additional confirmation of Ogston’s principle (4, 5).
The authors are grateful to Dr. J. C. R. Li of the Department of Mathematics, Oregon State College, for statistical evaluation of the data obtained, and to Mr. Richard C. Thomas for technical assistance.

SUMMARY

A study of the isotopic distribution in glutamic acid derived from CH$_3$C$^{14}$OCOOH or CH$_3$C$^{14}$OOH in yeast reveals that this amino acid is formed predominantly via the citric acid cycle. From acetate, in which virtually no growth of yeast occurred, there was extensive cycling; from pyruvate, in which growth was appreciable and glutamic acid was formed in large amounts, the data suggested a fairly direct series of reactions leading to glutamic acid, with much less cyclic activity than was displayed with acetate.

Whereas aspartic acid was previously shown to be formed without extensive equilibration with fumaric or other symmetrical C$_4$ acids, the equal distribution of isotope in carbons 2 and 3 of glutamic acid indicated that the C$_4$ condensing partner was symmetrically labeled. The significance of this observation is discussed with respect to the mechanism of the condensations believed to occur within the citric acid cycle.

BIBLIOGRAPHY

CONVERSION OF ACETATE AND PYRUVATE TO GLUTAMIC ACID IN YEAST
Chih H. Wang, Bert E. Christensen and Vernon H. Cheldelin


Access the most updated version of this article at [http://www.jbc.org/content/201/2/683.citation](http://www.jbc.org/content/201/2/683.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/201/2/683.citation.full.html#ref-list-1](http://www.jbc.org/content/201/2/683.citation.full.html#ref-list-1)