CONVERSION OF ACETATE AND PYRUVATE TO ASPARTIC ACID IN YEAST*

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Recent investigations by Ehrensvärd et al. (1) have revealed that the carbon skeleton of aspartic acid in Torula yeast can arise readily from administered acetate. With C\textsuperscript{13}H\textsubscript{3}C\textsuperscript{14}OOH, the aspartic acid formed during growth contained C\textsuperscript{14} equally distributed between the two carboxyl groups, and most of the C\textsuperscript{13} equally distributed between the 2 central carbon atoms. In the preceding papers (2, 3) the utilization of CH\textsubscript{3}C\textsuperscript{14}OOH and CH\textsubscript{3}C\textsuperscript{14}OCOOH by bakers' yeast has been compared, and have revealed that the incorporation of radioactivity into yeast protein is greater from pyruvate than from acetate.

Although the major energy-yielding pathway of pyruvate metabolism appears to proceed through “active” acetate as an intermediate, points of divergence are known to exist in anabolic processes (4). The fixation of CO\textsubscript{2} or its equivalent by pyruvate (5, 6) is one example. Since the extent to which pyruvate and acetate may follow a common pathway in the synthesis of yeast protein is unknown, the present study has been designed to establish this point: similar routes of metabolism would be reflected in similar patterns of distribution of activity in amino acids derived from carbonyl-labeled pyruvate and carboxyl-labeled acetate. The results described below indicate that, although aspartic acid from the acetate yeast bore the same C\textsuperscript{14} distribution as that reported by Ehrensvärd et al. (1), pyruvate produced a high degree of labeling in the amino carbon, suggesting that the Wood-Werkman (5) or some similar reaction (6) was operative to a major extent.

EXPERIMENTAL

Use was made of the yeast fractions prepared previously (2, 3), in which CH\textsubscript{3}C\textsuperscript{14}OOH and CH\textsubscript{3}C\textsuperscript{14}OCOOH were administered as sole carbon sources to cultures of Fleischmann's bakers' yeast. The pure radioactive copper aspartate obtained was diluted with inert aspartic acid as desired for the following degradation studies: (1) combustion to carbon dioxide for total

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radioactivity; (2) ninhydrin decarboxylation for the sum of α- and β-carboxyl groups; (3) preferential decarboxylation at 35° with chloramine-T for the radioactivity of the individual carboxyl groups (the α-carboxyl is discharged 4 times as rapidly as the β-carboxyl (1)); (4) combustion of the 2,4-dinitrophenylhydrazone of acetaldehyde (representing the methylene and amino carbons) obtained by means of sodium hypochlorite oxidation of aspartic acid (7); and (5) combustion of iodoform obtained directly by means of sodium hypoiodite oxidation of aspartic acid (for the methylene carbon). In the sodium hypoiodite oxidation, an amount of radioactive copper aspartate calculated to furnish about 100 c.p.m. per counting sample was diluted with inert aspartic acid to 1 mM. The mixture was treated at 60° for 12 hours in a tightly closed flask with 0.8 ml. of 50 per cent KOH and 15 ml. of 10 per cent iodine in 10 per cent KI solution. At the end of this period the iodoform crystals were collected in a sintered glass crucible and recrystallized from a methanol-water mixture.

Radioactivity of carbon was determined as barium carbonate in the conventional manner; counting data were corrected for background and self-absorption. The radioactivities of the various fractions or carbon atoms of aspartic acid were expressed on the basis of 1 mM of aspartic acid.

RESULTS AND DISCUSSION

The intramolecular distribution of activities in aspartic acid from 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 aspartic acid resulted in C₁⁴ incorporation exclusively in the carboxyl groups, in agreement with the observations of Ehrensvärd et al. (1). These workers have pointed out that it is possible to explain the equal carboxyl labeling in aspartic acid through operation of the citric acid cycle; Weinhouse and Millington (8) have also provided evidence for cyclic activity in Fleischmann's yeast during the oxidation of acetate. However, during growth, when oxalacetate is being removed by conversion to aspartic acid, some other means must be available to supply C₄ acids. An extended reversal of the cycle is ruled out, since the fixation of radioactive metabolic CO₂ into C₄, C₆, or C₈ acids would produce isotopic labeling in the central carbon atoms of aspartic acid. Therefore, if the cycle is to operate as postulated, it appears necessary to supply the C₈ acids through a preliminary tail to tail condensation of acetate. Such a condensation (9, 10) would provide the observed C₁⁴ distribution in aspartic acid, even in the absence of Krebs cycle activity. The C₄ acids, once formed, might equilibrate to some degree with pyruvate, as postulated by Cutinelli et al. (11) for Escherichia coli; in this way metabolic CO₂ from C¹³H₈C¹⁴OOH used in their yeast growth experiments would be
fixed into the $C_4$ carboxyls, contributing $^{13}$C as well as $^{14}$C to these groups (1).

In contrast to the foregoing picture, aspartic acid from pyruvate possessed a high degree of central carbon labeling. In the aerobic sample, the amino carbon alone bore 55 per cent of the total activity of the aspartic acid molecule. If this carbon atom is assumed to correspond to the carbonyl of the administered pyruvate, it then appears that fixation of a single carbon unit occurred, such as might be expected from the action of the "malic" enzyme (6) or the Wood-Werkman reaction (5). Labeling in the carboxyl groups, on the other hand, suggests that some of the aspartic acid was produced via the "active" acetate pathway, although a part (equivalent to $0.36 \times 10^6$ counts) could have arisen from cycling of the $C_4$ acids formed by $C_2-C_1$ fixation. The somewhat higher activity in the $\beta$-carboxyl over the $\alpha$-carboxyl group can be explained by $\beta$ fixation of metabolic $CO_2$ or its equivalent into $C_4$ acids, followed by transamination. (No excess isotope could remain in the $\alpha$-carboxyl after Krebs cycle activity, since this group would be discharged in the degradation of $\alpha$-keto-glutarate and would reappear as a randomly labeled product of fumarate oxidation.) Finally, the presence of a small amount of isotope in the methylene carbon can be ascribed to equilibration of the central carbons

<table>
<thead>
<tr>
<th>Group</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerobic acetate</td>
</tr>
<tr>
<td></td>
<td>c.p.m.*</td>
</tr>
<tr>
<td>Whole molecule</td>
<td>1.17</td>
</tr>
<tr>
<td>$\alpha$- + $\beta$-COOH</td>
<td>1.13</td>
</tr>
<tr>
<td>$\alpha$-COOH (Ehrensvård et al. (1))</td>
<td>0.60</td>
</tr>
<tr>
<td>$\beta$-COOH (Langheld (7))</td>
<td>0.61</td>
</tr>
<tr>
<td>Methylene + amino C (Langheld (7))</td>
<td>0</td>
</tr>
<tr>
<td>Methylene C§</td>
<td>0</td>
</tr>
<tr>
<td>Amino C (difference)</td>
<td>0</td>
</tr>
</tbody>
</table>

* All samples counted as BaCO$_3$, corrected for background and self-absorption, and expressed as counts per minute per millimole of degradation product oxidized.
† By ninhydrin decarboxylation.
‡ Specific activity expressed as counts per minute per 2 mm of C (equivalent to 1 mm of aspartic acid).
§ By NaO$I$ oxidation followed by combustion of CH$_4$; see the experimental section.
of the C₄ acids arising from C₅-C₁ condensation, either directly through fumarate or after operation of the cycle.

Each of the processes just described would lead to some isotope incorporation in the β-carboxyl group. A comparison of the observed total radioactivity in this group with that calculated to exist is therefore of interest. Thus, (1) in the aerobic sample, after C₅-C₁ condensation, the value of 0.36 × 10⁶ c.p.m. in the methylene group of aspartic acid is an index of randomization between the 2 central carbon atoms. The remaining 2.80 − 0.36 = 2.44 × 10⁴ counts in the amino group will be accompanied by 0.62 × 10⁴ c.p.m. in the β-carboxyl, based on the relative specific activities of the pyruvate and CO₂ (2). (2) Since all randomization, cycling, and C₅-C₁ condensation will place equal activity in the two carboxyl groups, it may be assumed that the observed value of 0.80 × 10⁵ in the α-carboxyl would be duplicated in the β-carboxyl. The total β-carboxyl activity (calculated) should therefore be 0.62 + 0.80 = 1.42 × 10⁵ c.p.m. This is in fair agreement with the value of 1.19 × 10⁵ observed. For the anaerobic sample, the agreement (1.32 × 10⁶ calculated, 1.28 observed) is much better. In view of the fact that the separate determination of activities in the carboxyl groups depends upon comparative rates of decarboxylation by the reagents used, it would appear that the agreement between the calculated and observed values is satisfactory, and that the suggested schemes for pyruvate conversion to aspartic acid represent a plausible interpretation of the data.

In the aerobic sample, if no cycling of the C₄ acids formed by C₅-C₁ condensation occurred, the apparent extent of this condensation was (2.80 + 0.36)/5.08 = 62 per cent; if cycling occurred (up to the maximum represented by 0.36 × 10⁴ c.p.m. in the methylene group), then the fraction would be (2.80 + 0.36 + 0.36 (2))/5.08 = 76 per cent. Anaerobically, the corresponding values were 41 to 61 per cent. It is also clear that equilibration of pyruvate with C₄ acids, and of the latter with each other, was not extensive in the pyruvate yeast. Only 0.36/(0.36 + 2.80) = 11 per cent of the carbonyl pyruvate isotope became distributed between the 2 central carbon atoms of aspartic acid during aerobic growth, assuming that no cycling ensued following C₅-C₁ condensation, or up to (0.36 + 0.36 (2))/(2.44 + 0.36 (4)) = 28 per cent with cycling. In the anaerobic sample the corresponding values for equilibration range between 25 and 50 per cent. It seems likely that the direct formation of aspartic acid was favored by the high concentrations of pyruvic acid present.

The quantitative differences observed here between acetate and pyruvate metabolism are being studied further with respect to other amino acids, particularly glutamic acid and tyrosine. These differences, with observations on the mechanisms involved, will be the subject of future publications.
The intramolecular distribution of radioactivity has been studied in aspartic acid from bakers’ yeast metabolizing CH$_3$C$^{14}$OOH and CH$_3$C$^{14}$OOCOOH. With acetate as substrate, the C$^{14}$ was contained exclusively in the carboxyl groups, equally distributed. A C$_2$–C$_2$ condensation was postulated to produce C$_4$ acids.

With pyruvate, a high degree of amino carbon labeling was observed. This was consistent with the concept of C$_2$–C$_1$ unit condensation, according to a Wood-Werkman or “malic” fixation scheme. The isotopic distribution indicated that 62 to 76 per cent of the radioactivity in aspartic acid arose by this route aerobically from pyruvate, and 41 to 61 per cent anaerobically.

**BIBLIOGRAPHY**

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