Transamination Reactions in
*Saccharomyces fragilis*

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SUMMARY: A dialysed cell-free extract of *Saccharomyces fragilis* Jörgensen was found to catalyse the transfer of the amino group from alanine, aspartic acid, isoleucine, leucine, norleucine, methionine, ornithine, phenylalanine, tryptophan, tyrosine or valine to α-ketoglutaric acid with the production of glutamic acid and the corresponding keto acid. The transaminase reactions leading to the formation of alanine, aspartic acid, phenylalanine or tryptophan from glutamic acid and the corresponding keto acid were also catalysed by the yeast extract. The addition of pyridoxal phosphate to the incubation mixture increased the rate of transamination between α-ketoglutaric acid and any one of the following amino donors: aspartic acid, leucine, methionine, phenylalanine, tryptophan or tyrosine.

The rate of formation of glutamic acid from α-ketoglutaric acid and leucine was most rapid at pH 8, but the rate of amino transfer from aspartic acid did not vary with pH over the range 6.0–9.0. The equilibrium constant for the aspartic-glutamic transamination reaction was found to be 2.2 at pH 7.8 and 37°.

The transfer of the amino group from aspartic acid or leucine to α-ketoglutaric acid was not inhibited by any one of five antibiotics tested, but was partially inhibited by 10⁻³m-semicarbazide.

Since the discovery of transamination reactions by Braunstein & Kritzmann (1937) opinion concerning the range and importance of such reactions in biological systems has undergone a cyclic change. The early studies by these two workers indicated that many amino acids were capable of transferring their amino groups to an acceptor such as α-ketoglutarate. Later work by Cohen (1939, 1942) seemed to restrict transaminase activity to two reactions involving glutamic and aspartic acids in one case and glutamic acid and alanine in the other. For several years transaminase reactions were considered to play only a limited role in the formation and interconversion of amino acids. Then Roine (1947) demonstrated that a direct transfer of the amino groups of valine, leucine, and isoleucine to α-ketoglutaric acid was catalysed by extracts of *Torulopsis utilis*, and Feldman & Gunsalus (1950) showed that dried cell preparations of *Escherichia coli*, *Pseudomonas fluorescens* and *Bacillus subtilis* catalysed the formation of glutamate from α-ketoglutarate and any one of thirteen amino acids, the amino acids utilizable varying slightly with the organism. Cammarata & Cohen (1950) showed that twenty-two amino acids other than aspartic acid, glutamic acid and alanine can participate in transamination in pig heart, liver and kidney tissues. Hird & Rowsell (1950) demonstrated that similar transaminase enzymes are present in rat liver mito-

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chondria, and that additional enzymes (Rowell, 1951) are present which utilize keto acids other than α-ketoglutarate as amino acceptors. Fincham (1951) demonstrated that dialysed extracts of Neurospora crassa catalysed some ten transamination reactions.

Studies on the concentration of aspartic and glutamic acids within the cell by Saccharomyces fragilis Jørgensen suggested that transaminase reactions might be involved in the internal metabolism of these dicarboxylic acids. The present work was therefore undertaken to determine the range of transaminase systems in S. fragilis and their relative importance in the metabolism of this organism.

**METHODS**

**Organism and enzyme preparations.** Saccharomyces fragilis Jørgensen from the Centraalbureau voor Schimmelcultures, Baarn, was used for the preparation of transaminase extracts. The yeast was grown in approximately 150 ml. of liquid medium in Roux bottles for 17 hr. at 25°C. The medium consisted of (%, w/v): NH₄H₂PO₄, 0·1; (NH₄)₂HPO₄, 0·1; KH₂PO₄, 0·2; Marmite, 0·01; 1% (v/v) of a trace element solution (20·0 g. MgSO₄·7H₂O; 1·0 g. NaCl; 0·5 g. FeSO₄·7H₂O; 0·5 g. MnSO₄·3H₂O; 0·05 g. CuSO₄·5H₂O; 10 ml. 0·1N-H₂SO₄; per litre of solution). Glucose was sterilized separately and was added to the autoclaved medium to a final concentration of 4% (w/v). After incubation the cells were harvested, washed once with 0·066~KH₂PO₄ and once with distilled water. In the earlier experiments the washed cells were treated with 10 vols. of cold (4°C) acetone, washed with acetone and ether and the powder stored in a desiccator over H₂SO₄ or CaCl₂. The acetone powder (50 mg.) was rubbed up with 0·1M-phosphate buffer (pH 7·8; 1·0 ml.) and the extract dialysed with stirring against 0·02~KCl at 1–2°C for 6–8 hr. The debris was removed by centrifugation at 1000 g and the clear supernatant fluid tested for transaminase activity. Storage at −15°C preserved the activity of the extracts for 1–2 weeks.

In the later part of this work, a Hughes press (Hughes, 1951) was used for the preparation of the cell-free extracts. Alumina powder (McIlwain, 1948) was mixed with an equal (wet) weight of yeast and the mixture crushed in a press previously cooled to −15°C. The resulting material was extracted with 0·05M-phosphate buffer (pH 7·8; 5 ml./g. wet wt. cells) and the extract dialysed and centrifuged as before.

**Assay for transaminase activity.** The formation of glutamic acid was demonstrated qualitatively by paper chromatography. Whatman no. 3 paper was spotted with 1–2 μl. of test solution and the chromatogram developed with butanol/water/acetetic acid (5:4:1) for 8–15 hr. by the descending technique (Consden, Gordon & Martin, 1944). In some instances, where the amino acids under study had widely different RF values, ascending chromatograms were developed for 2 hr. with phenol saturated with water (Hird & Rowell, 1950).

Glutamic acid and aspartic acid were estimated manometrically by the methods of Gale (1945) and Krebs (1950) respectively.

In qualitative work, incubations were made in 15 ml. centrifuge tubes con-
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containing 0.3 ml. enzyme solution, 10 μmole L-amino acid (20 μmole if the DL-isomer was used), 10 μmole α-ketoglutarate and 0.5 ml. 0.1M-phosphate buffer (pH 7.8) containing approximately 20 μg. pyridoxal phosphate/ml., in a total volume of 1.0 ml. Three controls were included in each set of experiments: one without amino acid, one without keto acid, and one with enzyme and buffer only, the amino acid and keto acid solution being replaced by 0.1 ml. 0.1M-phosphate buffer (pH 7.8). The tubes were flushed with nitrogen and closed with rubber stoppers. Incubation was at 37° for 1, 3 or 16 hr. The reaction was stopped by placing the tubes in boiling water for 3 min. The precipitate was centrifuged down and the clear supernatant used for a chromatogram.

For quantitative experiments, enzyme and buffer containing pyridoxal phosphate as above were placed in the centre compartment of a Warburg vessel and amino acid and/or keto acid in the side bulb. After gassing with nitrogen and equilibrating at 37° the contents were mixed. At the end of the incubation the pH was adjusted to 4.5 with N-HCl and the remaining traces of reaction mixture washed from the side bulb into the centre compartment with sufficient 0.2M-acetate buffer (pH 4.5) to bring the volume to 2.5 ml. The vessels were then placed in boiling water for 3 min. to inactivate the enzyme. After cooling, 0.5 ml. of a suspension of Clostridium welchii was placed in the side bulb and the glutamic acid estimated manometrically.

When aspartic acid was to be determined, the incubation was carried out in the same manner but the inactivation was modified by using 0.1M-acetate buffer (pH 5.0) to bring the volume of the reaction mixture to 2.0 ml. The suspension of Clostridium welchii was placed in the main compartment of the Warburg vessel so that any glutamic acid present in the sample was decarboxylated during the equilibration period. The other components of the aspartic acid test, i.e. 0.1 ml. α-ketoglutarate, 0.2 ml. partially purified horse heart transaminase and 0.05 ml. freshly distilled aniline were placed in the side bulb. The determination then followed the method of Krebs (1950).

RESULTS

Survey by chromatography of possible amino donors

Feldman & Gunsalus (1950) used paper chromatography in their studies on the transaminase systems of Escherichia coli, Pseudomonas fluorescens and Bacillus subtilis. Difficulties arose when a similar technique was applied to work with dried cells or crude extracts of Saccharomyces fragilis since this yeast contains a variety of amino acids in the free state within the cell, with especially high concentrations of glutamic acid and alanine. Any extract of these cells contains amounts of glutamic acid which interfere in the interpretation of a qualitative method such as chromatography. By growing the organism in a simple ammonium salt medium (Taylor, 1947) the concentration of glutamic acid within the cell was decreased but was still too high for clearly defined results. However, dialysis against 0.02M-KCl for 6–8 hr. was found to decrease the glutamic acid content of the extract below the level required to
give a colour with ninhydrin. A further difficulty was encountered when it was found that appreciable amounts of glutamate were produced in mixtures containing \( \alpha \)-ketoglutarate but no amino acid. The extract was shown to contain a glutamic dehydrogenase capable of reducing 1-35 \( \mu \)mole triphosphopyridine nucleotide/ml. extract/2.5 min., which probably accounts for glutamate formation from \( \alpha \)-ketoglutarate in the absence of added amino donors. This activity could be greatly diminished, and in some cases eliminated, by further dialysis of the extract for 24 hr., presumably by removal of the triphosphopyridine nucleotide. Other control mixtures in which
\( (a) \ \alpha \)-ketoglutarate was omitted, \( (b) \) the enzyme preparation was heated to 100°, \( (c) \) \( \alpha \)-ketoglutarate was replaced by oxalacetate or pyruvate, showed no trace of glutamate formation.

Twenty-two amino acids were tested both qualitatively and quantitatively for ability to transfer their amino group to \( \alpha \)-ketoglutarate in the presence of the dialysed yeast extract. A spot corresponding to glutamic acid was formed when aspartic acid, leucine, phenylalanine, methionine, tryptophan or tyrosine was the amino donor.

**Quantitative estimation of transamination**

The results of the quantitative experiments in which the glutamate formed was measured manometrically are given in Table 1. The experiments were carried out with extracts of acetone powders and with extracts of whole cells prepared with the Hughes press.

**Acetone powder extracts.** After incubation for 1 hr., aspartic acid, leucine, phenylalanine, methionine, tyrosine, tryptophan, isoleucine and valine (in order of decreasing activity) were found to act as amino donors with \( \alpha \)-ketoglutarate as amino acceptor. After 3 hr. quantitative, but not qualitative, differences were observed. It was possible, however, by increasing the time of reaction to 16 hr. to confirm the presence of the two transaminases of much lower activity which had isoleucine and valine as substrates. In some preparations there seemed to be some evidence of slight alanine, ornithine, threonine and arginine transaminase activities but the results were variable.

**Hughes press extracts.** The absence of an alanine-glutamate transaminase from the acetone powder extracts was unexpected. It seemed possible that the acetone treatment received by the cells was too severe and inactivated the enzyme which catalysed this reaction. Therefore the survey was repeated using an extract prepared from whole cells crushed in the Hughes press at \(-15\)° (Table 1). In addition to the eight amino acids given above, alanine, norleucine and ornithine were able to transfer their amino groups. In general the activities observed with Hughes press extracts were higher than with extracts of acetone powders.

**Effect of pyridoxal phosphate on rate of transamination**

Chromatographic experiments showed that the amount of glutamate formed by transamination from leucine, aspartic acid, phenylalanine, methionine, tyrosine or tryptophan was increased in the presence of pyridoxal
Table 1. Transaminase activity in extracts of Saccharomyces fragilis

1 ml. of incubation mixture contained: 10 µmole amino acid (L-isomer), 10 µmole α-ketoglutarate, 0·3 ml. dialysed yeast extract, 0·1 M-phosphate buffer (pH 7·8) containing 20 μg. pyridoxal phosphate/ml. Incubation was at 37° in N₂ and glutamate was estimated on boiled, deproteinized mixtures. All figures are corrected for glutamate formed (<0·5 µmole/mg. extract N) in the absence of an added amino donor.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Hughes press extract</th>
<th>Acetone powder extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr.</td>
<td>16 hr.</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>3·65</td>
<td>25·14</td>
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<tr>
<td>L-Citrulline</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glycine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-Histidine</td>
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</tr>
<tr>
<td>L-Hydroxyproline</td>
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<td>0</td>
</tr>
<tr>
<td>L-Norleucine</td>
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<td>—</td>
</tr>
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<td>L-Isoleucine</td>
<td>0·6</td>
<td>2·11</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>5·67</td>
<td>16·84</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>2·79</td>
<td>10·2</td>
</tr>
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<td>L-Ornithine</td>
<td>0</td>
<td>0·85</td>
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<tr>
<td>L-Phenylalanine</td>
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<td>11·0</td>
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<tr>
<td>L-Proline</td>
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<td>0</td>
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<tr>
<td>DL-Serine</td>
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<td>0</td>
</tr>
<tr>
<td>DL-Threonine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L-Tryptophan</td>
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<td>6·39</td>
</tr>
<tr>
<td>L-Tyrosine</td>
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<td>7·3</td>
</tr>
<tr>
<td>L-Valine</td>
<td>0·52</td>
<td>3·89</td>
</tr>
</tbody>
</table>

phosphate. That the degree of activation is not the same in each system is seen from the quantitative data for aspartic acid, leucine and tryptophan (Table 2). Tryptophan gave the greatest response, no glutamate being formed in the absence of pyridoxal phosphate. Aspartic acid showed the smallest increase in activity, while the leucine system showed an intermediate response. Partial resolution of the transaminase(s) into apo-enzyme and coenzyme during preparation of the extract could account for this increase in activity in the presence of pyridoxal phosphate.

Table 2. Effect of pyridoxal phosphate on rate of transamination

1 ml. of incubation mixture contained: 10 µmole amino acid, 10 µmole α-ketoglutarate, 0·1 M-phosphate buffer (pH 7·8), 0·3 ml. dialysed yeast extract, 20 µg. pyridoxal phosphate as indicated. Incubation was at 37° for 1 hr. in N₂.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Without pyridoxal phosphate</th>
<th>With pyridoxal phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Aspartic acid</td>
<td>4·82</td>
<td>6·84</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>2·23</td>
<td>4·82</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0</td>
<td>1·88</td>
</tr>
</tbody>
</table>
Glutamic acid as an amino donor in transaminase reactions

Evidence for the reversibility of four of the transamination reactions, aspartic-, alanine-, phenylalanine- and tryptophan-glutamic acid, was obtained chromatographically (Fig. 1). Only these four reactions, for which the equivalent keto acids were readily available, were tested. In each case, material corresponding to the amino acid was formed on incubation of the Hughes press extract with glutamic acid and the keto acid.

![Diagram of paper chromatogram](image)

Fig. 1. Diagrammatic representation of paper chromatogram showing formation of alanine, phenylalanine, tryptophan and aspartic acid from glutamic acid and the corresponding keto acid catalysed by *Saccharomyces fragilis* extract. One ml. of incubation mixture contained: 10 μmole glutamic acid, 20 μmole keto acid, 0.8 ml. dialysed Hughes press extract, 20 μg. pyridoxal phosphate, 0.05 M-phosphate buffer (pH 7.8). Incubation was for 16 hr. at 37° in N₂. Reaction stopped by heating at 100° for 3 min. Supernatant fluid used to spot chromatogram. Solvent system: butanol-acetic acid.

Attempts to obtain transamination using oxalacetate or pyruvate as amino acceptor and α-amino butyrate, leucine, methionine, ornithine, phenylalanine, tryptophan, tyrosine or valine as amino donor were unsuccessful. It would appear that only transaminase reactions in which either α-ketoglutarate or glutamate is a substrate are catalysed by the dialysed yeast extract under the conditions used.
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Conditions affecting the α-ketoglutarate-leucine transamination

The two most active transaminase systems, aspartic acid + α-ketoglutarate and leucine + α-ketoglutarate, were studied in more detail.

Rate of glutamate formation. A mixture containing leucine, α-ketoglutarate and dialysed acetone powder extract was incubated and the glutamate formed measured after various intervals. Under the conditions used, the amount of glutamate formed increased continuously during the whole course of the experiment (Fig. 2). Initially there was a rapid rate of transamination which gradually decreased but did not cease. Failure to reach a constant glutamate concentration was probably due to non-enzymic breakdown of the α-ketoisocaproic acid which was formed in the reaction, resulting in a shift of the equilibrium to the glutamate side.

Effect of temperature. The rate of the leucine-α-ketoglutarate reaction was found to increase linearly over the range 25–37° with a temperature coefficient of 1.98. Above 37° the rate of increase was diminished.

Effect of pH. The influence of pH on transamination varies with the source of enzyme and with the particular transaminase reaction studied. Lichstein & Cohen (1945) found a very sharp peak at pH 8.5 for the aspartic-glutamic transaminase activity of intact cells of Escherichia coli while the corresponding reaction with dried cells or cell-free extracts of Streptococcus faecalis showed little variation over the range pH 6.0–9.5 (Lichstein, Gunsalus & Umbreit, 1945). In extracts of Torulopsis utilis the reaction between glutamic acid and oxalacetic acid has a maximum activity at pH 7.8 (Roine, 1947). Fincham (1951) found the amino group transfer from isoleucine, ornithine or phenylalanine to α-ketoglutarate with dialysed neurospora extracts to be most rapid at pH 8, that from aspartate at pH 9 and from alanine at pH 5.5–6.0. Amino group transfer from leucine to α-ketoglutarate by a Hughes press extract of Saccharomyces fragilis, tested over a pH range 6.0–9.0, was most rapid at pH 8 (Fig. 3). Neither the formation of glutamate from aspartate + α-keto-glutarate nor the formation of aspartate from glutamate + oxalacetate was sensitive to changes in pH value over the same range. At no point on the curve was there more than a 5% variation from the mean value.

Effect of antibiotics. The leucine-glutamic acid transaminase and the aspartic-glutamic acid transaminase were not inhibited by aureomycin, chloramphenicol, penicillin, streptomycin or terramycin at 100 μg/ml. Both reactions were, however, partially inhibited by 10⁻³M-semicarbazide, the former system by about 60% and the latter by about 45%.

Reversibility of the aspartic-glutamic acid transaminase system

There is chromatographic evidence for the reversibility of this reaction (Fig. 1); the equilibrium has been measured quantitatively by approaching it from both sides (Fig. 4). The initial rate of reaction A (glutamic acid → aspartic acid) is much more rapid than that of reaction B (aspartic acid → glutamic acid), an observation consistent with the results of Cohen (1940) who used
animal tissues and of Roine (1947) who used a *Torulopsis utilis* extract. Curve A shows a maximum formation of aspartic acid after 2 hr. followed by a slight decrease, probably due to the instability of oxalacetic acid at the pH value of the experiment. In the case of the reverse reaction (B) the spontaneous decomposition of oxalacetic acid results in an increase in the amount.

Fig. 2. Rate of glutamate formation from leucine + α-ketoglutarate. One ml. of incubation mixture contained: 10 μmole L-leucine, 10 μmole α-ketoglutarate, 0.2 ml. dialysed yeast extract, 0.1 M-phosphate buffer (pH 7.8) containing 20 μg. pyridoxal phosphate/ml. Incubated at 37° in N₂.

Fig. 3. Effect of pH value on glutamate formation from leucine + α-ketoglutarate. Incubation mixture as in Fig. 2 except for variation in pH value as indicated. Incubated at 37° in N₂ for 2 hr.

Fig. 4. Transamination between (A) glutamate and oxalacetate, (B) aspartate and α-ketoglutarate. One ml. of incubation mixture contained: 7 μmole L-amino acid, 14 μmole keto acid, 0.2 ml. yeast extract, 0.1 M-phosphate buffer (pH 7.8) containing 20 μg. pyridoxal phosphate/ml. Incubated at 37° in N₂.
of glutamate formed. Calculation of the equilibrium constant for the reaction gives a value of
\[
K = \frac{[\text{aspartate}][\alpha\text{-ketoglutarate}]}{[\text{glutamate}][\text{oxalacetate}]} = 2.2 \text{ at pH 7.8 and 37°,}
\]
a figure which is in reasonable agreement with that of 3.5 obtained by Cohen (1940) with purified pig heart enzyme.

I wish to thank Dr R. Davies and Dr E. F. Gale, F.R.S., for their interest in this work. I also wish to express my appreciation to the U.S. State Department for a Fulbright Fellowship during part of this investigation, and to the National Institute of Microbiology of the U.S. Public Health Service for a Predoctoral Fellowship during the remaining part. I am grateful to Dr R. E. Kallio, Dr E. V. Rowsell and Dr W. W. Umbreit for their gifts of indole-3-pyruvic acid, phenylpyruvic acid and pyridoxal phosphate, respectively.

REFERENCES


(Received 21 October 1954)