TRANSAMINATION OF AMINO ACIDS WITH GLYOXYLIC ACID IN BACTERIAL EXTRACTS1

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The formation of glycine by transamination of glyoxylic acid and amino acids has been reported with rat liver preparations (Meister et al., 1952; Nakada and Weinhouse, 1953) and with dialyzed juice of tobacco leaves (Wilson et al., 1954). Wright (1951) demonstrated that glyoxyxlate replaced glycine in the growth of a Neurospora mutant and Weinhouse and Friedmann (1951) showed that glyoxyxlate was rapidly converted to glycine in the intact rat. These observations suggest that transamination reactions with glyoxyxlate may be widespread in nature.

The present paper reports on the transamination of amino acids with glyoxyxlate by an unidentified strain of Pseudomonas (Campbell, 1954). Cell extracts of this organism transaminate alanine, aspartic acid, and glutamic acid with glyoxyxlate, giving rise to glycine and the corresponding keto acids. Asparagine and glutamine undergo transamination and deamidation, forming glycine, ammonia, and keto acids.

EXPERIMENTAL METHODS

Culture. Pseudomonad strain 2RCC-1 was grown in a medium containing proteose peptone, 1 per cent; yeast extract, 0.5 per cent; K2HPO4, 0.1 per cent in distilled water; pH 7.4. After incubation for 18 hr at 30 C on a shaker, the cells were harvested by centrifugation and washed three times with 0.01 M phosphate buffer, pH 8.0.

Cell-free extract. Extracts were prepared by grinding with alumina at 0 C as described earlier (Campbell, 1954), except that the paste was extracted with 0.01 M phosphate buffer, pH 8.0. The clear straw-colored supernatant, obtained by centrifugation at 10,000 X G for 15 min, was used as the enzyme. Extracts stored at -10 C retained transaminase activity for several weeks.

Transaminase assay. Except where noted, reaction mixtures usually contained extract, 50 mg; glyoxyxlate, 20 μM; amino acid, 10 μM; and pyridoxal phosphate, 20 μg; in a final volume of 0.01 M phosphate buffer; 2 ml; pH 8.0. After incubation for 60 min at 30 C, the reaction mixtures were placed in a boiling water bath for 5 min, cooled, and centrifuged to remove the precipitated protein. The supernatant was used for analysis. Appropriate controls showed no evidence of nonenzymatic transamination during the incubation period employed.

Methods. Glycine was identified by ascending and descending paper chromatography on Whatman No. 1 paper in three solvents: tertiary butanol:water:formic acid (70:15:15) (Meister et al., 1952); ethanol-ammonia (95 ml 95 per cent ethanol:5 ml conc. NH4OH) (Berry et al., 1951); and phenol saturated with pH 12 buffer (paper previously buffered at pH 12) (McFarren, 1951). Papers were developed with ninhydrin.

For quantitative determination of glycine, the method of Alexander et al. (1945) as modified by Christensen et al. (1951) was employed.

Keto acids were identified by paper chromatography of their 2,4-dinitrophenylhydrazones in terti-amy1 alcohol:ethanol:water (5:1:4) (Altman et al., 1951). The method of Cavallini et al. (1949) was used for quantitative estimation of the keto acids.

Asparagine was determined by the method of Krebs (1950); glutamine, glutamic and aspartic acids by decarboxylation with Clostridium perfringens (Krebs, 1948; Meister et al., 1951).

Ammonia was determined by nesslerization after aeration into sulfuric acid, or by adsorption (pH 7.0) and elution from "permutil" (Folin and Bell, 1917). Protein content of the extracts was estimated colorimetrically by the method of Lowry et al. (1951).

RESULTS

The data presented in table 1 show that of a wide variety of amino donors tested only alanine,
glyoxylic acid. For each of the active donors, the corresponding keto acid was identified by chromatography of the 2,4-dinitrophenylhydrazones.

Quantitative data on the synthesis of glycine from glyoxylic acid by transamination are presented in Table 2. It may be seen that there is a quantitative relationship between the amount of amino donor disappearing, the formation of the corresponding keto acid and the synthesis of glycine. In addition to transamination, asparagine and glutamine undergo deamidation, giving rise to ammonia.

Thoroughly dialyzed extracts stored at –20°C for 10 weeks had no transaminase activity in the absence of pyridoxal phosphate. Full activity could be restored by the addition of 20 μg per ml of pyridoxal phosphate.

The optimum pH range for transaminase activity was 8.0 to 9.0. Figure 1 shows the effect of pH on transamination between glyoxylic and glutamic acids.

**DISCUSSION**

The present investigation has demonstrated that extracts of Pseudomonad synthesize glycine from alanine, aspartic acid, glutamic acid, asparagine and glutamine by transamination with glyoxylic acid. The reaction may be visualised as follows:

\[
\text{amino acid} + \text{glyoxylic acid} \rightleftharpoons \text{glycine} + \text{keto acid}
\]

The finding that asparagine and glutamine are deamidated in the presence of glyoxylic acid is in agreement with the report of Meister et al. (1952) for the deamidation of these compounds with keto acids by rat liver preparations. Meister (1954a, 1954b) and Meister and Frazer (1954)
have reported that the transamination-deamidation reaction involves two steps: (a) transamination of glutamine and asparagine with keto acid, yielding amino acid and \( \alpha \)-ketoglutarate and \( \alpha \)-ketosuccinamate, respectively; and (b) the subsequent hydrolysis of these compounds to ammonia and \( \alpha \)-ketoglutarate and oxalacetate. While there is as yet no evidence for the formation of \( \alpha \)-ketoglutarate and \( \alpha \)-ketosuccinamate by the bacterial extracts employed in the present study, it is possible that they are intermediates in the transamination-deamidation reactions reported here. This point is under investigation and will be reported on later.

**SUMMARY**

Cell free extracts of Pseudomonad strain 2RCC-1 synthesize glycine from alanine, aspartic acid, glutamic acid, asparagine and glutamine by transamination with glyoxylic acid. Asparagine and glutamine also undergo deamidation, forming ammonia. The transamination reactions are pyridoxal phosphate dependent, requiring 20 \( \mu \)g per ml for full activity. The optimum pH range for activity is from 8.0 to 9.0.

**REFERENCES**


Wright, B. E. 1951 Utilization of glyoxylic and glycic acids by a Neurospora mutant requiring glycine or serine. Arch. Biochem. and Biophys., 31, 332-333.