Transamination Reactions of Mammalian Cells in Tissue Culture*

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A wide variety of mammalian cells in culture have been shown to require thirteen amino acids for survival and growth (1-4). The nonessential amino acids, not supplied in the medium, are synthesized by the cells from other components of the medium. Glucose and glutamine (5) have been shown to serve as sources of carbon. Since transamination is an important reaction in the interconversion and biosynthesis of amino acids, it became of interest to examine the range of transaminase systems in cultured cells. Both a human cervical carcinoma cell (strain HeLa) and a mouse fibroblast (strain L) are here shown to have active transaminating systems leading to the formation of alanine, glutamic and aspartic acids, glycine, phenylalanine, and tyrosine from their corresponding α-keto analogues.

EXPERIMENTAL

Growth and Preparation of Cell Suspensions and Cell-free Extracts— Cultures of the HeLa cell and mouse fibroblast were grown as cellular layers adherent to the glass surface of 1-liter Blake bottles overlaid with the basal medium containing thirteen amino acids, eight vitamins, salts, and glucose (6). The medium was supplemented with 5 per cent dialyzed human serum in the case of the HeLa culture, and with 5 per cent dialyzed horse serum in the case of the L strain.

After 5 to 7 days of incubation at 37°, the medium was decanted, and the adherent cell layer washed twice with cold Krebs-Ringers salt solution (7) and harvested by scraping into the salt solution. Cell-free extracts were prepared by sonic disruption of a 20 per cent cell suspension in 10 to 15 ml. of distilled water in a Raytheon 9 kc. sonic oscillator for 10 minutes, followed by centrifugation at 20,000 × g for 30 minutes. Such preparations contained 15 to 20 mg. of protein per ml. and maintained their transaminase activity for several weeks when stored at -15°. The protein contents of the extracts were determined both by a modification of the procedure of Lowry (8) and by the micro-Kjeldahl method. The experiments reported here were carried out with these extracts, preliminary experiments having shown them to be more active than whole cells.

Assay of Transaminase Activity—The reaction mixtures contained usually 10 μmoles of keto acid, 20 μmoles of amino acid, 25 μg. of crystalline pyridoxal phosphate, phosphate buffer (0.1 m) of pH 7.5 to 8.0, and 1 ml. of enzyme preparation in a total volume of 3 ml. After incubation for 3 hours at 37° the reaction was stopped by heating in a boiling water bath for 5 minutes; the sample was then cooled, and 0.2 ml. of 3 M acetate buffer (pH 4.9) was added. The precipitated protein was removed by centrifugation, and an aliquot of the supernatant fluid was removed for amino acid assay. Results are expressed in terms of μmoles of amino acid formed per ml. of enzyme preparation.

Assays of Amino Acids—Quantitative assay of amino acids formed was performed chromatographically as previously described (9). In addition, L-glutamic acid, L-glutamine, and L-aspartic acid were determined by means of the acids by decarboxylation with lyophilized Clostridium perfringens (10).

Glycine was estimated colorimetrically by the method of Alexander et al. (11), as modified by Christensen et al. (12). Most of the L-amino acids used were obtained commercially and were tested chromatographically for purity. Crystalline pyridoxal phosphate was kindly provided by Drs. E. A. Peterson and H. A. Sober, National Cancer Institute, Bethesda, Maryland.

α-Keto acids were obtained commercially. Chromatographic examination of their 2,4-dinitrophenylhydrazones showed that they were free from other keto acids. Sodium glyoxylate was synthesized by a modification of the method of Metzler et al. (13). We are indebted to Dr. Leon Levintow, National Institutes of Health, for samples of phenylpyruvic acid and p-hydroxyphenylpyruvic acid. Isonicotinic acid hydrazide (isoniazid) was a gift of Dr. Benjamin Proccott, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

RESULTS

The ability of cell-free extracts of the HeLa cell to mediate the transfer of the α-amino group of 17 amino acids to pyruvate, oxaloacetate, α-ketoglutarate, glyoxylate, phenylpyruvate, and p-hydroxyphenylpyruvate is shown in Table I. Similar experiments with extracts of the mouse fibroblast are summarized in Table II. Glutamic acid was the most active amino donor in both cell lines. In contrast, threonine and cysteine were consistently either minimally active or completely inactive as amino donors. It is of interest that whereas the amino groups of lysine, histidine, and methionine were activated by the extracts derived from the L cells, these amino acids were relatively inactive in transamination mediated by extracts derived from the HeLa cells. NH₃ was completely inactive with both cell lines.

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† H. Eagle, K. A. Piez, V. I. Oyama, and R. Fleischman, unpublished observations.
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Glutamine is essential for the growth of these cultures (3), and is used by the cells for the synthesis of both protein and nucleic acid (5, 15). In a purified rat liver system, Meister et al. (16) have shown that glutamine rather than glutamic acid was active in transamination. However, Tables I and II show that glutamic acid was 9 to 16 times more effective than glutamine as an amino donor in transamination mediated by extracts from the L and HeLa cells, respectively. These results suggest that in these systems glutamine is first deaminated (5), and the resulting glutamic acid transaminated with the appropriate α-keto acid; the rate-limiting step in transamination presumably is the deamination of glutamine.

Great variability was observed in the rates of transamination between different amino acids and α-keto acids (cf. Fig. 1). Thus, with pyruvate as the amino acceptor there were marked differences in rates between glutamate and isoleucine, and between isoleucine and valine when α-ketoglutarate was employed as the acceptor. Similar results were obtained with HeLa extracts. With both cell lines transamination reactions leading to the formation of arginine, valine, and leucine were either minimal or absent. The D isomers of the amino acids which were.

One of the most active transaminase systems studied was that catalyzing the formation of aspartic acid from oxaloacetate and glutamic acid. Aspartic acid is nutritionally nonessential for all the culture cells studied in this laboratory, and is presumably synthesized by the cell from oxaloacetate produced via the tricarboxylic acid cycle (14).
tested, i.e. D-leucine, N-glutamate, N-phenylalanine, N-alanine, and d-aspartic acid were completely inactive as amino donors.

Transamination with Dipeptides—It has been shown that certain dipeptides can substitute for their corresponding essential amino acids for the growth of the mouse fibroblast and the HeLa cell (17). Table III shows that dipeptides were not so effective as amino donors as the component amino acids in the formation of glutamate catalyzed by extracts of the I. cell. The reaction mixtures after incubation contained the component amino acids of the dipeptides, indicating that the extracts contained dipeptidases. It seems probable that hydrolysis of the dipeptides is a necessary precondition to their activity as amino donors.

Effects of Pyridoxal Phosphate and Isoniazid—Although pyridoxal phosphate has been shown to be a coenzyme of transaminases (18, 19), no effect was noted upon its addition in the various reactions studied with dialyzed cell-free extracts of the tissue cells. Presumably, the pyridoxal phosphate is firmly bound to the apoenzyme in the enzyme preparation employed. Isoniazid, which has been found to inhibit a number of enzyme systems including transaminases in bacteria (20, 21), had little or no effect on the transaminases of the tissue cell extracts studied.

**SUMMARY**

1. Cell-free extracts of both the HeLa cell and mouse fibroblast form aspartic and glutamic acids, alanine, glycine, phenylalanine, and tyrosine by transamination of the corresponding a-keto acids with a variety of amino acids.

2. Glutamic and aspartic acids, leucine, isoleucine, and alanine were most active as amino donors. Other amino acids tested were weak or inert.

3. Glutamic acid was 9 to 16 times more effective than glutamine as an amino donor, and was the most active amino donor tested.

4. Several dipeptides were found to function in transamination reactions equally as effectively as the component amino acids. Since the extracts contained active dipeptidases, it is not clear whether the peptides per se or the constituent amino acids were the active compounds.

5. Pyridoxal phosphate and isoniazid had no appreciable effect on the transaminases.

**REFERENCES**

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