Alteration in the Amino Acid Content of Yeast During Growth Under Various Nutritional Conditions

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Yeast cells grown under optimal and suboptimal concentrations of biotin were analyzed for the amino acid content of their soluble pool and cellular protein. Optimally grown yeast cells exhibited a maximum amino acid content after 18 hr of growth. Biotin-deficient cells were depleted of all amino acids at 26 and 43 hr, with alanine, arginine, aspartate, cysteine, glutamate, isoleucine, leucine, lysine, methionine, serine, threonine, and valine being present in less than half the concentration observed in biotin-optimal cells. At early time intervals, the amino acid pool of biotin-deficient yeast contained lower concentrations of all amino acids except alanine. After more prolonged incubation, several amino acids accumulated in the pool of biotin-deficient yeast, but citrulline and ornithine accumulated to appreciable levels. The addition of aspartate to the growth medium resulted in a decrease in the amino acid content of biotin-optimal cells but caused a marked increase in the concentration of amino acids in biotin-deficient cells. The pools of biotin-deficient yeast grown in the presence of aspartate displayed a marked reduction in every amino acid with the exception of aspartate itself. These data provide evidence that the amino acid content of yeast cells and their free amino acid pools are markedly affected by biotin deficiency as well as by supplementation with aspartate, indicating that aspartate plays a major role in the nitrogen economy of yeast under both normal as well as abnormal nutritional conditions.

Our knowledge of the biosynthesis and utilization of amino acids in yeast has progressed to a point where the origins and pathways of formation of many of the amino acids are well documented (6, 8, 10, 13). However, several questions still remain with regard to the reactions through which inorganic nitrogen is assimilated into key amino acids and with regard to their subsequent contribution of their nitrogen and carbon to other amino acids. In particular, the importance of aspartate as a key factor in these processes has not been fully accepted, despite strong evidence from nutritional and isotope labeling studies which clearly show that aspartate and alanine contribute carbon or nitrogen, or both, to a number of other amino acids as well as to purines, pyrimidines, and other cellular constituents (2–4, 6–8, 10, 11,

13, 15, 17, 18). Other reports in the literature imply that glutamate is the only amino acid involved in the assimilation of inorganic nitrogen and the transfer of its amino nitrogen to other amino acids (9, 17).

Stokes and Gunness (19) determined the amino acid content of yeast grown in both complex and defined media. They reported that variation in the nutritional environment could appreciably alter the amino acid content of yeast, as opposed to earlier reports which indicated that the amino acid composition of microorganisms was independent of the nutritional environment as long as required factors were not omitted (1, 7, 21). Moat and Emmons (14) confirmed the earlier demonstration by Koser, Wright, and Dorfman (11) that aspartate was the only single amino acid which could significantly stimulate the growth of yeast in a medium deficient in biotin. Since Ahmad and Rose (3) and Ahmad, Rose, and Garg (4) had demonstrated that the protein content and the content of amino nitrogen of biotin-deficient yeast was altered by biotin-sparing sub-

stances, we decided that determination of the changes in content of specific amino acids of yeast, grown under biotin-optimal and biotin-deficient conditions and in the presence and absence of aspartate, would provide important additional information as to the role of aspartate in the amino acid economy of yeast.

MATERIALS AND METHODS

Saccharomyces cerevisiae, Fleischmann strain 139, was grown in the defined medium utilized in earlier nutritional investigations (14) at an optimal biotin concentration of 0.2 ng/ml and at a deficiency level of 0.01 ng/ml. Cultures were grown for 18, 26, 43, 114, and 140 hr at 32 C, harvested by centrifugation, washed twice with distilled water, and resuspended in distilled water. The dry weight of cells was determined turbidimetrically by comparison with a standard curve. Samples for analytical determinations were taken from this suspension so as to provide 150 mg (dry weight) of cells for analysis of the amino acid content of the cells and amino acid pools.

The protein content of the yeast was determined by the micro-Kjeldahl method described by Spies (18). Amino nitrogen of the soluble pool was determined by the ninhydrin method outlined by Ballentine (5) with glycine as the standard.

The procedure utilized for amino acid analysis was as follows. The free amino acid pool was obtained by extracting 150 mg (dry weight) of cells in 10 ml of distilled water at 100 C for 10 min. The cells were then centrifuged, the supernatant fluid was removed, and the cells were washed with an additional 4 ml of boiling distilled water. The supernatant fluid from this washing was added to the original supernatant fluid, and the total volume was brought to 15 ml. The samples were frozen until analyzed. After extraction of the amino acid pool, lipids were removed from the cells by shaking in a mixture of chloroform and methanol (3:1, v:v) for 24 hr. The cells were removed from the solvent mixture by filtration, washed with ligroin, dried, and hydrolyzed by refluxing for 72 hr in 15 ml of 6 N HCl. The HCl was removed by repeated evaporation under vacuum; the samples were brought to a final volume of 15 ml with distilled water and frozen until analyzed.

Amino acid analysis was conducted with the Technicon Amino Acid Autoanalyzer system employing type B Chromobead resin, 8% cross-linked. The concentration of amino acids (in micromoles) was determined from the area under each peak by comparison with standard curves obtained with a known mixture of amino acids. The values presented represent the average of three determinations on each sample.

RESULTS

Alteration of amino acid composition with nutritional environment. Considerable differences were observed in the growth, total protein content, and the amino nitrogen content of the soluble pool of yeast grown in the presence of sub-

optimal concentrations of biotin as compared with yeast grown in the presence of optimal biotin (Fig. 1 and 2), as was previously observed by Ahmad and Rose (3) and Ahmad, Rose, and Garg (4). The overall effects of biotin deficiency and aspartate supplementation on the total protein and soluble amino nitrogen of yeast prompted us to investigate whether this was a general manifestation or whether it was related to the formation of specific amino acids.

Analysis of yeast cells after growth for periods ranging from 18 to 140 hr revealed that the amino
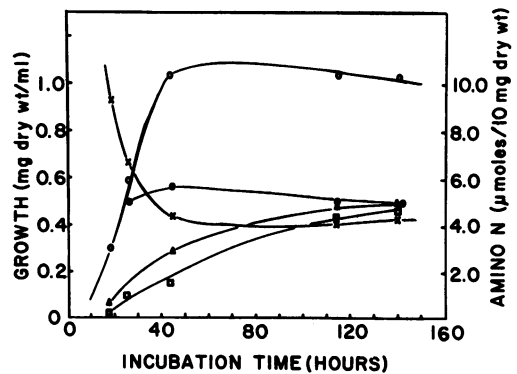

FIG. 1. *Growth and amino nitrogen content of the soluble pool of S. cerevisiae, Fleischmann strain 139, under various nutritional conditions. Symbols:* $\bigcirc$, *optimal biotin (0.2 ng/ml) with* NH_4^+ *as sole N source;* $\square$, *biotin deficient (0.01 ng/ml) with* NH_4^+ *as sole N source;* $\triangle$, *biotin deficient supplemented with 200 μg of aspartate per ml;* $\times$, *amino nitrogen of biotin-optimal pool;* $\bullet$, *amino nitrogen content of biotin-deficient pool.*
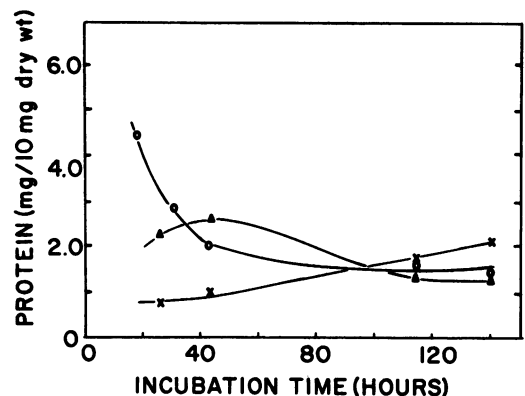

FIG. 2. *Variation in protein content of S. cerevisiae, Fleischmann strain 139, under different nutritional conditions. Symbols:* $\bigcirc$, *optimal biotin (0.2 ng/ml) with* NH_4^+ *as sole N source;* $\times$, *biotin deficient (0.01 ng/ml) with* NH_4^+ *as sole N source;* $\triangle$, *biotin deficient supplemented with 200 μg of aspartate per ml.*

acid content of cells grown with ammonia as the sole source of nitrogen in the presence of optimal biotin reached a peak at the earliest time interval tested (Fig. 3). Subsequently, a decline was observed in the concentration of every amino acid except cysteine. By comparison, cells analyzed after growth in the presence of limiting amounts of biotin were depleted of every amino acid. Alanine, arginine, aspartate, cysteine, glutamate, isoleucine, lysine, methionine, serine, threonine, and valine were reduced by more than 50% after 26 or 43 hr of growth (Fig. 3). In most instances, the greatest amino acid deficiency was observed at the earliest time interval at which sufficient amounts of biotin-deficient cells could be obtained for analysis (26 hr). Biotin-deficient cells also showed a decline in amino acid concentration with time, but this decline was more gradual, so that the amino acid levels of biotin-deficient and of biotin-optimal cells were very nearly equal by the end of 140 hr of growth.

The amino acid pools of optimally grown yeast were found to contain relatively high concentrations of alanine, arginine, and glutamate and lower, but significant, levels of the other amino acids (Fig. 3). With a few notable exceptions, the peak of amino acid concentration in the optimal pools was observed at the earliest time of harvest (18 hr) and gradually declined after prolonged

incubation. Alanine, which was present in high concentration initially, remained at this level throughout. Aspartate, which was present at relatively low concentration at 18 hr, increased to a peak at 43 hr and then declined.

The pools of biotin-deficient yeast displayed low concentrations of all amino acids except alanine. This amino acid appeared in almost the same concentration as in optimal pools at 26 and 43 hr and then declined. All of the other amino acids gradually rose in concentration until they equalled or exceeded the levels in optimal pools. Glutamate represented an obvious exception to this general finding, displaying an increase and then a decline so that obvious deficiency was observed at 140 hr.

Variation in pool content of arginine, citrulline, and ornithine. Citrulline, which was barely detectable in the pool of optimally grown yeast, was found to accumulate to a high level in the pool of biotin-deficient cells. Ornithine, which was present in detectable levels in the pool of optimally grown cells, also increased and then declined in the pool of deficient cells (Fig. 4). As described below, yeast grown in the presence of aspartate did not accumulate either citrulline or ornithine in the pools of biotin-deficient or optimally grown cells.
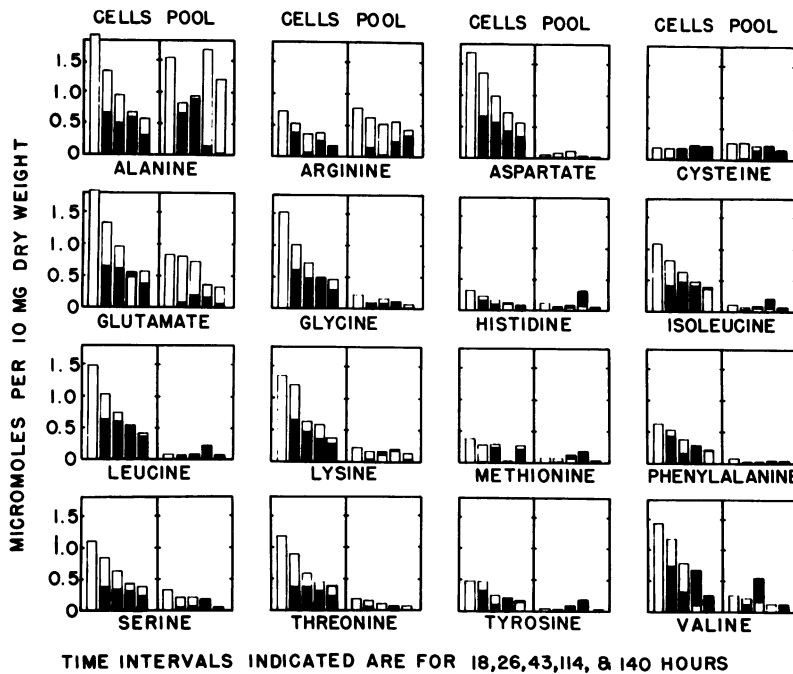
Effect of aspartate on amino acid composition

FIG. 3. *Amino acid composition of the cells and free amino acid pool of S. cerevisiae, Fleischmann strain 139. Values for yeast grown in the presence of optimal biotin (0.2 ng/ml) are shown by the open bars. Those for yeast grown in a biotin-deficient medium (0.01 ng/ml) are shown by the closed bars.*
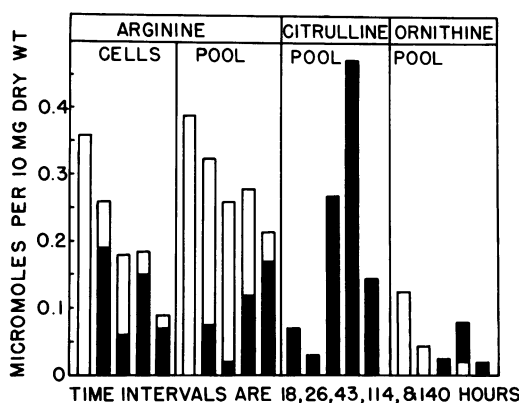
FIG. 4. *Effect of biotin deficiency on the pool levels of arginine, citrulline, and ornithine. Values for optimally grown yeast (0.2 ng/ml) are shown by the open bars. Those for biotin-deficient yeast (0.01 ng/ml) are shown by the closed bars.*

Growth of the yeast in a biotin-deficient medium supplemented with aspartate brought about even more pronounced differences in the overall content of amino nitrogen in the soluble pool as compared with yeast grown with ammonia as the sole source of nitrogen. The pool of optimally grown cells contained 0.68 μmole of amino nitrogen per mg (dry weight), whereas biotin-deficient pools contained 0.50 μmole of amino nitrogen per mg (dry weight). By comparison, the amino acid pools of biotin-deficient yeast grown in the presence of aspartate dropped to 0.14 μmole of amino nitrogen per mg (dry weight).

To obtain more detailed information on the extent of this effect, the amino acid content of yeast was compared after growth with and without aspartate as a supplementary source of nitrogen. Several interesting variations were observed in the cells and pools of both optimal and biotin-deficient yeast after growth in the presence of aspartate (Fig. 5). In the presence of aspartate, optimally grown cells displayed a reduced concentration of all amino acids with the exception of cysteine. Biotin-deficient cells contained higher concentrations of amino acids than did biotin-optimal cells after growth in the presence of aspartate. Also the concentrations of all amino acids equalled or exceeded those observed in biotin-deficient cells grown with ammonium sulfate as the sole source of nitrogen.

Under the conditions of biotin deficiency and with ammonium sulfate as the sole source of nitrogen, most amino acids accumulated in the pools to levels equalling or exceeding those of the pools of optimally grown yeast. Addition of aspartate to the growth medium resulted in a reduction of the pool level of many amino acids

and virtual disappearance of others from the biotin-deficient pools. Arginine, histidine, isoleucine, leucine, methionine, and valine dropped to undetectable levels in the pools of biotin-deficient cells grown in the presence of aspartate. Growth in the presence of aspartate did not markedly alter the amino acid pool content of biotin-optimal cells. Aspartate accumulated in high concentrations in the pool of biotin-deficient cells grown in the presence of aspartate but was unaltered in concentration in the pool of biotin-optimal cells.

DISCUSSION

The amino acid content of optimally grown yeast cells reached a peak of concentration at 18 hr and diminished with time over the balance of the period of observation. This coincides with the fact that the protein content of yeast is maximal at 18 hr and declines thereafter. The marked depletion of all amino acids in biotin-deficient yeast attests to the fact that biotin deficiency results in a general depression of amino acid production or protein synthesis, or both. However, the depletion of alanine, arginine, aspartate, cysteine, glutamate, isoleucine, leucine, lysine, methionine, serine, threonine, and valine by 50% or more implicates biotin more specifically in the control of the production of these amino acids or in their utilization for protein synthesis. Of this group, only lysine and serine were not required in the amino acid mixture which had been shown to stimulate the growth of biotin-deficient yeast (14). Aspartate is known to contribute directly to the biosynthesis of threonine, isoleucine, and methionine in yeast (2, 8, 10, 13, 23). It can also interchange carbon and nitrogen directly with glutamate via a combination of the tricarboxylic acid cycle and transaminase activity, and participates in the formation of arginine by contributing its amino nitrogen to citrulline to form arginosuccinate (8, 13). No obvious direct relationships exist between biotin or aspartate and the other amino acids which are depleted in biotin-deficient yeast. Biotin deficiency has long been known to result in a marked reduction in aspartate (10, 11, 13, 14), most likely as a result of a deficiency in the supply of dicarboxylic acids for its synthesis (16). Thus, the limited amounts of aspartate, threonine, methionine, and isoleucine in biotin-deficient cells and their rapid replenishment by the addition of aspartate is readily explained.

The depletion of arginine in biotin-deficient cells correlates directly with the accumulation of ornithine and citrulline in the amino acid pool. This can best be explained on the basis of dual effects of biotin deficiency on arginine biosynthesis. Aspartate nitrogen is required for the conver-
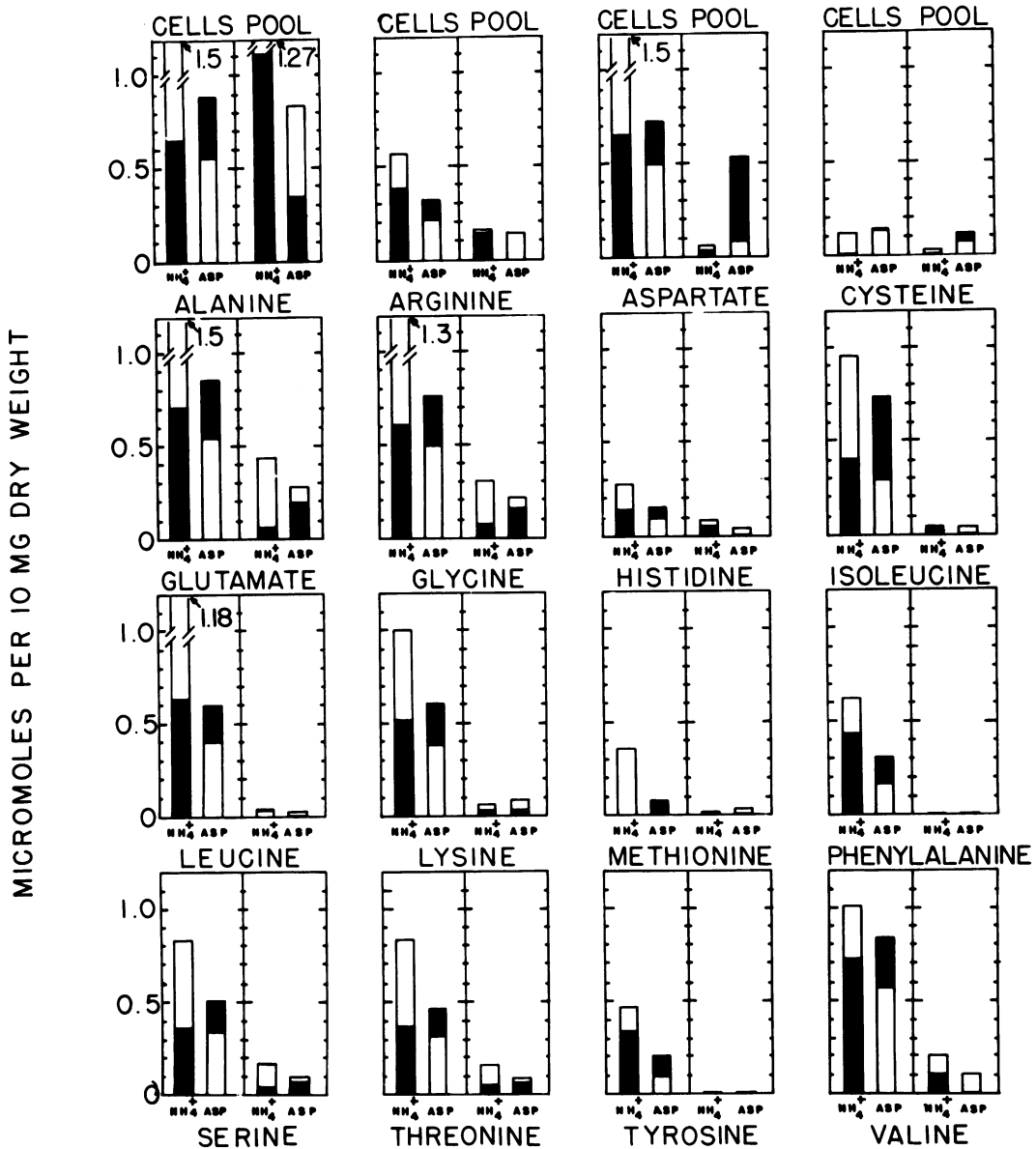
FIG. 5. *Effect of aspartate on the amino acid composition of optimally grown (0.2 ng/ml) and biotin-deficient (0.01 ng/ml) yeast cells and their free amino acid pools. Values for optimally grown yeast are shown by the open bars. Those for the biotin-deficient yeast are shown by the closed bars. Time of harvest was 26 hr. The aspartate concentration was 200 μg/ml.*

sion of citrulline to arginine (8, 13). Limitation-of the supply of aspartate would result in the accumulation of citrulline. Carbamyl phosphate synthetase has recently been shown to require biotin (24). Depletion of the supply of carbamyl phosphate would result in ornithine accumulation. It might be reasoned that citrulline should not accumulate under such conditions. However, it must be remembered that the yeast cells are growing under a deficiency state which permits

approximately one-third maximal growth. This permits partial activity of all systems whose synthesis or function is directly or indirectly related to biotin. A similar phenomenon occurs in purine biosynthesis in yeast grown under biotin deficiency in that both aminoimidazole ribonucleotide and hypoxanthine accumulate as a result of limitations at two different points in the pathway (15).

The reduced glutamate concentration in biotin-

deficient cells may also be explained on the basis of limiting aspartate, since it is rapidly increased in the presence of added aspartate (Fig. 5). The limited formation of glutamate under the conditions of biotin deficiency undoubtedly results from the deficiency of carboxylic acids. Lysine, which is derived from α-ketoglutarate in yeast (8, 13, 20), is also depleted in biotin-deficient yeast for the same reason. Aspartate serves as a source of additional carboxylic acids for glutamate and lysine synthesis under these conditions.

Reduction in the concentration of alanine, cysteine, serine, valine, and leucine in biotin-deficient yeast is less readily explained on the basis of direct or indirect relationships with biotin or aspartate. The one common feature relating these amino acids is the contribution of pyruvate to their carbon skeleton (8, 13). Inspection of the pool levels of these amino acids (Fig. 3) indicates that they are not significantly depleted in the pools of biotin-deficient yeast. This suggests that their depletion in the cell protein is the result of lack of utilization rather than through any serious limitation in their formation. Biotin deficiency may, therefore, be interpreted as imposing a condition of unbalanced growth by directly limiting the biosynthesis of certain amino acids, which, in turn, prevents the utilization of others even though they are formed in adequate amounts. The latter accumulate in the soluble pool. Upon addition of aspartate (or biotin), the formation of the limiting amino acids provides for additional growth and protein synthesis since the amino acid balance is at least partially restored. As noted in the growth curve (Fig. 1), however, biotin-deficient yeast cells appear to be in a prolonged exponential phase, explaining why there is continued activity even after several days, whereas in optimally grown cells maximal synthetic activity is essentially complete in 48 hr.

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