JOURNAL OF BACTERIOLOGY, Jan. 1972, p. 25-33

Vol. 109, No. 1
Printed in U.S.A.

Inorganic Nitrogen Assimilation in Yeasts: Alteration in Enzyme Activities Associated with Changes in Cultural Conditions and Growth Phase

KENNETH W. THOMULKA[1] AND ALBERT G. MOAT
Department of Microbiology, Hahnemann Medical College, Philadelphia, Pennsylvania 19102

Received for publication 15 July 1971

Ammonia assimilation has been investigated in four strains of *Saccharomyces cerevisiae* by measuring, at intervals throughout the growth cycle, the activities of several enzymes concerned with inorganic ammonia assimilation. Enzyme activities in extracts of cells were compared after growth in complete and defined media. The effect of shift from growth in a complete to growth in a defined medium (and the reverse) was also determined. The absence of aspartase (EC 4.3.1.1, L-aspartate-ammonia lyase) activity, the low specific activities of alanine dehydrogenase, glutamine synthetase [EC 6.3.1.2, L-glutamate-ammonia ligase (ADP)], and the marked increase in activity of the nicotinamide adenine dinucleotide phosphate-linked glutamate dehydrogenase (NADP-GDH) [EC 1.4.1.4, L-glutamate:NADP-oxidoreductase (deaminating)] during the early stages of growth support the conclusion that yeasts assimilate ammonia primarily via glutamate. The NADP-GDH showed a rapid increase in activity just before the initiation of exponential growth, reached a maximum at the mid-exponential stage, and then gradually declined in activity in the stationary phase. The NADP-GDH reached a higher level of activity when the yeasts were grown on the defined medium as compared with complete medium. The nicotinamide adenine dinucleotide-linked glutamate dehydrogenase (NAD-GDH) [EC 1.4.1.2, L-glutamate:NAD-oxidoreductase (deaminating)] showed only slight increases in activity during the exponential phase of growth. There was an inverse relationship in that the NADP-GDH increased in activity as the NAD-GDH decreased. The NAD-GDH activity was higher after growth on the complete medium. The glutamate-oxaloacetate transaminase (EC 2.6.1.1. L-aspartate:2-oxoglutarate aminotransferase) activity rose and fell in parallel with the NADP-GDH, although its specific activity was somewhat lower. Although other ammonia-assimilatory enzymes were demonstrable, it seems unlikely that their combined activities could account for the remainder of the ammonia-assimilatory capacity not accounted for by the NADP-GDH. The ability of aspartate to serve as effectively as glutamate as the sole source of nitrogen for the growth of yeasts apparently resides in their ability to utilize aspartate for amino acid biosynthesis via transamination.

It has been demonstrated in a variety of microorganisms that inorganic nitrogen may be assimilated into amino nitrogen via glutamate, alanine, aspartate, carbamyl phosphate, and glutamine, but no one organism utilizes all of these routes in an equal manner (15). Usually, a given organism will utilize one or two pathways predominantly, to the virtual exclusion of the others (9). Early observations showed that aspartate was superior to ammonia or other amino acids for the growth of yeasts (15). Much of the information in the literature supports the role of glutamate as the major route of ammonia assimilation in yeasts (3, 5, 11, 19), although few definitive studies

[1] Present address: Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19104.

have been conducted to substantiate this generalization. Both nicotinamide adenine dinucleotide-linked glutamate dehydrogenase (NAD-GDH) [EC 1.4.1.2, L-glutamate:NAD oxidoreductase (deaminating)] and nicotinamide adenine dinucleotide phosphate-linked glutamate dehydrogenase (NADP-GDH) [EC 1.4.1.4, L-glutamate:NADP-oxidoreductase (deaminating)] have been observed in yeasts at high specific activities (2, 10, 17), and it has been observed that two-thirds of the ammonia assimilated by yeast can be initially recovered as glutamate or glutamine (11). This leaves the question as to which of the assimilatory systems accounts for the remaining one-third of the nitrogen requirement.

We have conducted experiments in which amino acids and inorganic ammonia were first compared as nitrogen sources for the growth of *Saccharomyces cerevisiae*. Four strains of yeast were grown on a minimal and a defined medium, and the assimilatory enzymes were assayed after the cells had reached the stationary phase (K. Thomulka and A. G. Moat, Bacteriol. Proc. p. 37, 1969; ibid., p. 148, 1970). Based upon the results of these preliminary studies, more detailed experiments were conducted in which these enzymes were assayed at various intervals from time of inoculation through the exponential and stationary phases. The effect of transfer from minimal to complete medium (shift-up conditions) and from complete to minimal (shift-down conditions) were also investigated. These experiments permitted a comparison of the activity of enzymes concerned with nitrogen assimilation during all phases of growth and the effect of shifts in the nutritional environment of their activities.

MATERIALS AND METHODS

Organisms. Four strains of yeast were employed. *S. cerevisiae* 174/1d, a wild, haploid yeast, and *S. cerevisiae* 173/1a, an adenine-requiring mutant derived from strain 174/1d were obtained from the collection of B. Ephrussi (1949) through the courtesy of A. M. Srb, Cornell University, Ithaca, N.Y. *S. cerevisiae* Fleischmann strain 139, a standard strain which has been widely used in a variety of nutritional and metabolic studies (1), and *S. cerevisiae* strain X-1049-2B, a glutamate-oxaloacetate transaminase (GOT)-deficient mutant obtained from the collection of M. Ogur, University of Southern Illinois, Carbondale.

Media. The complete medium was composed of 2% glucose, 1% Difco yeast extract, 1% Difco peptone, and 0.5% KH_2PO_4. The defined medium used for strains 174/1d and Fleischmann 139 contained the following ingredients, in amounts per liter: ammonium sulfate, 0.3 g; glucose, 20.0 g; KH_2PO_4, 0.5 g; $CaCl_2$, 62.5 mg; $MgCl_2$, 20.0 mg; $MnCl_2$, 1.0 mg; $FeCl_3$, 0.5 mg; inositol, 3.0 mg; beta-alanine, 1.0 mg; pyridoxine hydrochloride, 20 μg; thiamine, 20.0 μg; nicotinic acid, 100 μg; and biotin, 1.0 μg. For strain 173/1a, the above medium was supplemented with 80.0 mg of adenine. The GOT-deficient strain (X-1049-2B) required the above medium supplemented with 200 mg of aspartate, 100 mg each of glutamate and tryptophan, and 80 mg each of histidine, arginine, methionine, threonine, lysine, uracil, and adenine. The *p*H of all media was adjusted to 5.6 before autoclaving for 15 min at 121 C.

Growth conditions. All cultures were grown at 32 C under aeration with compressed air passed through a glass-wool filter and distributed through a ground-glass sparger. Silicone antifoam (Dow Corning) spray was added, as required, to reduce foaming.

Variation of growth conditions. For the development of shift-up and shift-down conditions (transfer from defined to complete and from complete to defined medium) starter cultures in 15.0 ml of complete medium were inoculated from a stock culture and incubated overnight. The cells from these cultures were transferred to 4 liters of medium and grown overnight; the cells were harvested, washed twice with sterile distilled water, and transferred to 9.0 liters of fresh medium. For inoculation into complete medium, the entire 15 ml of the starter culture was transferred into the 4-liter cultures. For inoculation into defined medium, the cells were washed once with distilled water before inoculation. For transfer from the 4-liter to the 9-liter cultures, cells were harvested after 24 hr of incubation, washed twice in sterile distilled water, and suspended to 100 ml. At time of harvest, each liter contained 1.8 g (dry weight) of cells. A 15-ml portion was withdrawn for the zero-time sample, and the remainder was inoculated into the 9-liter carboy. The 9-liter cultures were incubated under aeration, and 2.0-liter samples were taken after 30, 75, 135, and 240 min of incubation. A final 1.0-liter sample was taken after 24 hr of incubation.

The use of a large inoculum for the 9-liter cultures made it possible to obtain samples during the early phases of growth which would provide a sufficient quantity of cells to prepare extracts. In addition, the use of a large inoculum allowed the cultures to enter the exponential phase rapidly and to reach the stationary phase within a time span that would permit convenient sampling during the entire period of active growth.

Cell harvesting. The samples were immediately centrifuged; cells were combined, washed twice with distilled water and once with 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (*p*H 8.0), and immediately disrupted.

Cell disruption. A 5-g (wet weight) amount of cells and 5.0 g (wet weight) of washed 5-μm glass beads were suspended to 25 ml in Tris buffer. The cell-bead suspension was transferred to a stainless steel sonic oscillating chamber equipped with an external cooling jacket. Alcohol at −4 C was passed

through the jacket to maintain low temperature during sonic treatment. The cells were disrupted with a Branson Sonifier for two 5-min periods, with a 30-sec interval to allow for cooling. The broken-cell suspension was removed from the chamber and centrifuged at 27,000 × *g* for 30 min. The lipid pellicle was removed, and the supernatant fluid was decanted and stored at 4 C for assay.

For disruption in a Duall tissue homogenizer, 10.0 g (wet weight) of cells was suspended to 20.0 ml in Tris buffer and ground in a size D homogenizer cooled by immersion in an ice bath. The homogenizer was turned by a Talboy laboratory stirring motor at full speed for 20 min. The resulting mixture was centrifuged as for the sonically treated cells.

To prepare extracts with the X-press, all the cells harvested from 6.0 liters of a stationary-phase culture were suspended to 35 ml in Tris buffer. The cell paste was added to a precooled X-press cell at −35 C. The X-press was then immersed for 30 min in a −35 C alcohol bath to insure complete freezing of the cells. The pressure cell was then placed in a Carver hydraulic press and the pressure was raised to 24,000 psi. The broken cells were collected, slowly thawed, and centrifuged as above.

Assay methods. Protein determinations were made by the method of Lowry et al. (14) with bovine serum albumin used as a standard.

All enzymatic reactions were conducted at 32 C. The reaction vessel was immersed in a 32 C water bath or placed in a water-jacketed chamber of the spectrophotometer. All extracts were prepared and assayed on the same day as cell harvest. Unless otherwise specified, all reactions were monitored on a Beckman DU spectrophotometer modified with a Gilford electronic optical density conversion system and recorder.

Aspartase was measured by the method of Williams and Lartigue (22) by measuring fumarate production at 240 nm or by the microdiffusion method of Ternberg and Hershey (21) in which the reaction mixture was the same as that employed by Williams and Lartigue (22).

The nicotinamide adenine dinucleotide phosphate-linked alanine dehydrogenase (NADP-ADH) and the NAD-GDH and NADP-GDH were measured spectrophotometrically by the method described by Burk and Pateman (5). The reaction mixture consisted of: ammonium sulfate, 4.0 μmoles; reduced nicotinamide adenine dinucleotide phosphate or reduced nicotinamide adenine dinucleotide, 1.7 μmoles; Tris buffer, 100 μmoles; and pyruvate or glutamate, 22.0 or 6.4 μmoles, respectively; in a final volume of 3.0 ml.

Glutamine synthetase was assayed by the method of Kohlaw et al. (13). Threonine deaminase [EC 4.2.1.16 threonine hydrolase (deaminating)] was assayed by the method of Holzer et al. (11). The GOT and glutamate-pyruvic acid transaminase (GPT) [EC 2.6.1.2, L-alanine:2-oxoglutarate amino-transferase)] were measured by coupling with the NADP-GDH. The 3.0-ml reaction mixture contained 1.7 μmoles of reduced nicotinamide adenine dinucleotide phosphate; ammonium sulfate, 4.0 μmoles; pyridoxal-5'-phosphate, 0.04 μmole; Tris buffer, 100 μmoles at *p*H 7.5; oxalacetate or pyruvate, 4.0 μmoles; and extract, 0.01 ml.

Polyacrylamide-gel electrophoresis was carried out by using a 7% gel and the method of Fincham and Stadler (7).

RESULTS

Amino acids as sole sources of nitrogen for the growth of yeasts. Since aspartate is one of the few amino acids which have been shown to be superior to ammonia as a sole source of nitrogen for the growth of yeast (15), the relative effectiveness of aspartate, glutamate, and ammonium sulfate as sole nitrogen sources in the defined medium were compared. The nitrogen content of the medium was the same (0.0227 M) in each case. When *S. cerevisiae* 173 1/a was grown on medium containing these sources of nitrogen, aspartate was found to be equal to ammonia as the sole source of nitrogen, whereas glutamate was inferior (Fig. 1). With aspartate, the generation time was equal to that with ammonium sulfate (3.0 hr). With glutamate, the generation time increased to 4.65 hr and the total amount of growth was less. None of the individual nitrogen sources were as efficient as the complete medium (Fig. 1). These preliminary results suggested that aspartate could serve as an efficient source of nitrogen for growth and that aspartase might represent a significant route of inorganic nitrogen assimilation in yeast.

Enzyme levels in cells harvested at stationary phase. Studies were conducted to

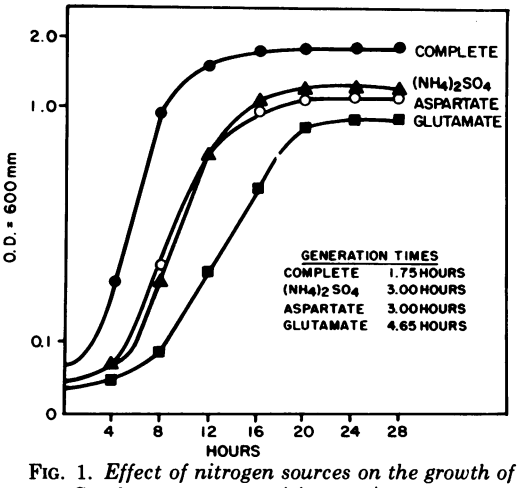

FIG. 1. *Effect of nitrogen sources on the growth of yeast. Saccharomyces cerevisiae 173/1a was grown on a defined medium with 0.0227 M ammonium sulfate, aspartate, or glutamate as the sole nitrogen source, and the growth was compared with that on complete medium. Optical density was measured at 600 nm in a Bausch & Lomb Spectronic 20 spectrophotometer.*

determine and compare the specific activities of several enzymes of potential significance in ammonia assimilation after overnight growth. By using the X-press, extracts were prepared from cells grown to stationary phase on both the complete and the defined media. Table 1 presents the specific activities obtained. Each value is the average of at least three separate determinations, each made on a different extract.

In each strain, the NADP-GDH was derepressed after growth on the defined medium. The NAD-GDH was derepressed and higher in activity than the NADP-GDH after growth on the complete medium for three of the strains tested. In all strains there was a trend toward an inverse relationship between the NAD-GDH and NADP-GDH. These tendencies are amplified by the data obtained with extracts taken from cells harvested at earlier stages in the growth cycle. When grown on the complete medium, the NADP-GDH was repressed and lower in activity than the NAD-GDH, whereas the reverse was true after growth on the defined medium. The activity of the NADP-ADH was low as compared with the NADP-GDH and was simultaneously repressed. GPT activity was very low and only occasionally demonstrable. The GOT activity was derepressed when the yeasts were grown on the defined medium and repressed on the complete medium in a manner comparable with the NADP-GDH and NADP-ADH. Threonine deaminase activity tended to follow that of the NAD-GDH, but not in any concerted manner. Glutamine synthetase levels were so low that no clear-cut comparison could be made with other enzyme activities. Aspartase was lacking in all of the strains tested.

Variation in enzyme activities with growth phase and cultural conditions. To determine whether cells harvested during different phases of growth varied with regard to the activity of ammonia assimilation enzymes, and whether the cultural conditions gave rise to alterations in the level of these activities, cells were grown under conditions which would permit determination of the levels of ammonia assimilation enzymes at various phases of growth in defined and complete media. The effect of shift-up and shift-down conditions were also investigated. Figures 2 to 5 present the specific activities of the various enzymes after shift-up and shift-down conditions for each of the four strains investigated. In all strains and at all times during growth, aspartase activity was undetectable. Efforts were made to detect aspartase activity by means of ammonia evolution (21) and fumarate production (22) over a *p*H range of 6.0 to 8.5, with and without β-mercaptoethanol, glutathione, and cysteine. Both crude extracts and extracts which had been dialyzed and desalted on Sephadex G-50 or treated with $MnCl_2$ to remove nucleic acids were used. Separate experiments were conducted on extracts from cells grown in

TABLE 1. *Effect of cultural conditions on the specific activities of ammonia assimilation enzymes in four strains of Saccharomyces cerevisiae*

Organism	Medium	Enzyme[a]							
		NADP-GDH	NADP-ADH	NAD-GDH	GOT	GPT	Threonine deaminase	Aspartase	Glutamine synthetase
S. cerevisiae 173/1a[b]	Complete	484	13	163	46	11	74	0	0
	Defined	1,984	33	270	150	0	39	0	4
S. cerevisiae, 174/1d (wild type)	Complete	176	8	249	83	10	121	0	3
	Defined	790	33	559	150	8	67	0	0
S. cerevisiae Fleischmann 139	Complete	194	26	598	37	0	45	0	0
	Defined	975	20	112	115	0	15	0	0
S. cerevisiae X-1049-2B[c]	Complete	130	4	148	0	0	331	0	0
	Defined	651	20	236	0	0	32	0	0

[a] Results are expressed as 10^{-4} μmoles of product formed per min per mg of protein. Abbreviations: NADP-GDH, nicotinamide adenine dinucleotide phosphate glutamate dehydrogenase; NADP-ADH, nicotinamide adenine dinucleotide phosphate-alanine dehydrogenase; NAD-GDH, nicotinamide adenine dinucleotide-glutamate dehydrogenase; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvic acid transaminase.

[b] Adenine-requiring strain.

[c] GOT-deficient strain.

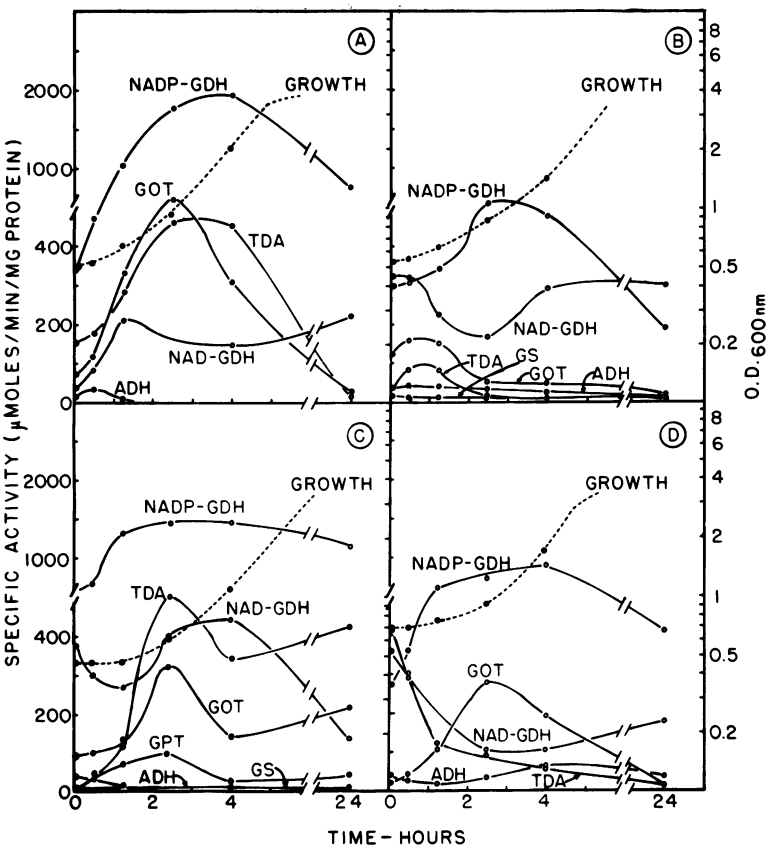

FIG. 2. *Effect of cultural conditions on the specific activity of ammonia assimilation enzymes and GOT in Saccharomyces cerevisiae 173/1a. The organism was transferred from (A) complete to complete, (B) complete to defined, (C) defined to defined, and (D) defined to complete medium. NADP-GDH, NADP-glutamate dehydrogenase; ADH, alanine dehydrogenase; NAD-GDH, NAD-glutamate dehydrogenase; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase; TDA, threonine deaminase; GS, glutamine synthetase.*

both complete and defined media in which the concentration of glucose had been reduced to 0.5% to determine whether glucose repression might account for low aspartase activity. In experiments in which fumarate production was used as an indicator of aspartase activity, the addition of orthoiodosobenzoate (0.002 M) did not enhance detection of aspartase, although fumarase was inhibited. Aspartase activities were compared in extracts obtained by means of the X-press, the Branson Sonifier, and the Duall tissue homogenizer. Under all of the conditions employed, aspartase activity was undetectable, despite the fact that the extracts were fully active with respect to other enzymatic activities.

Glutamine synthetase was present in very low levels under all cultural conditions. Its activity was somewhat higher at mid-exponential phase. However, there was no apparent relationship between the activities of the NAD-GDH, threonine deaminase, and glutamine synthetase as had been reported by earlier investigators (10, 11, 13).

The GOT levels rose in conjunction with the increase in activity of the NADP-GDH. The GPT activity was present at much lower levels of activity than the GOT and remained relatively constant throughout the growth cycle.

In all strains and at all times, whether grown on the complete or defined medium, NADP-GDH had the highest activity of any ammonia assimilation enzyme. The NAD-GDH was generally lower and its activity was inversely related to that of the NADP-GDH. The NAD-GDH activity was lowest when the NADP-GDH displayed the highest specific activity. The NADP-GDH increased markedly during

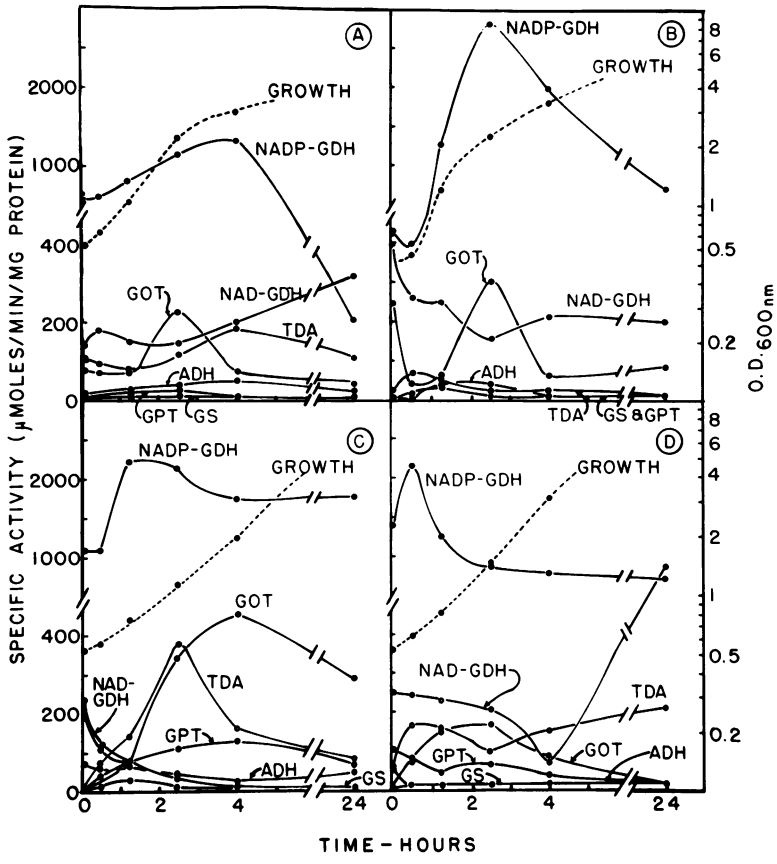

FIG. 3. *Effect of cultural conditions on the specific activity of ammonia assimilation enzymes and GOT in Saccharomyces cerevisiae 174/1d. Conditions and abbreviations as for Fig. 2.*

the early exponential phase of growth, rapidly reached a peak, and declined to much lower levels at stationary phase. The GOT, although substantially lower in activity, followed this same pattern in all but the GOT-deficient mutant.

The NADP-ADH activity of each strain remained at a low level throughout the growth cycle. The activity increased slightly when cells were grown on defined rather than on complete medium.

Threonine deaminase did not consistently follow the NAD-GDH activity as Holzer et al. had found (11). Threonine deaminase was more active after growth on the complete medium than after growth on the defined medium. In several cases, there was a sharp increase in activity during the early exponential phase followed by a decline at later time intervals (see Fig. 2–4).

In the GOT-deficient mutant (Fig. 5), GOT, GPT, glutamine synthetase, and aspartase were undetectable at significant levels at any point in the growth cycle. On transfer from complete to complete medium, the NAD-GDH and NADP-GDH followed an unusual pattern as compared with the other strains (Fig. 5). The NADP-GDH increased but did not reach the level of activity observed with the other strains. Furthermore, the activity remained relatively constant throughout growth. The NAD-GDH activity increased markedly at the beginning of exponential growth, declined sharply, and then gradually increased at the stationary phase (Fig. 5A). In the shift-down transfer (Fig. 5B), the NADP-GDH was lower in activity than on direct transfer from defined medium. The NAD-GDH was higher in activity under these conditions.

DISCUSSION

Initial experiments indicated that aspartate was superior to ammonia or glutamate as a source of nitrogen for the growth of yeast. This led to experiments designed to determine

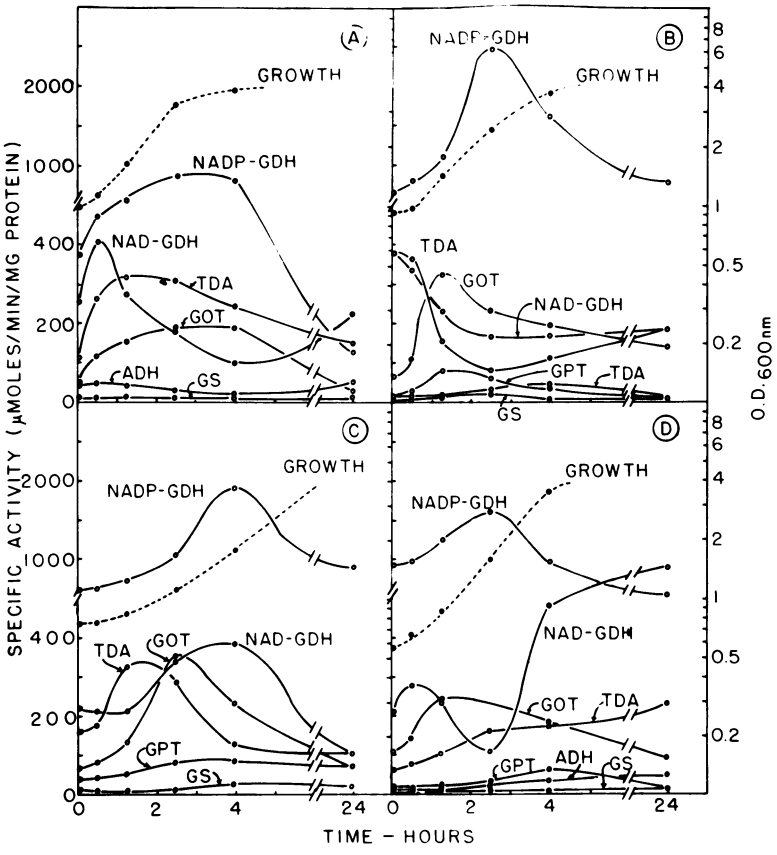

FIG. 4. *Effect of cultural conditions on the specific activity of ammonia assimilation enzymes and GOT in Saccharomyces cerevisiae Fleischmann strain 139. Conditions and abbreviations same as Fig. 2.*

whether inorganic nitrogen was assimilated directly into aspartate by the enzyme aspartase. An early report (8) indicated that aspartase was present in whole yeast cells. Polakis and Bartley (17) also demonstrated a weakly active aspartase in cell-free extracts of yeast. However, we were unable to detect aspartase in any of the yeasts investigated. Because the extracts were fully active with respect to all other enzymatic activities, our inability to detect aspartase is considered to be due to its absence and not due to denaturation during the extraction procedures. The demonstration by Haehn and Leopold (8) of ammonia evolution from aspartate by whole cells of yeast must have resulted from the transfer of aspartate nitrogen to either glutamate or threonine and the subsequent deamination of these amino acids. The fact that yeasts grow well on aspartate as the sole source of nitrogen must reside in their ability to transfer aspartate nitrogen to other amino acids via GOT. Our results suggest that, in yeasts, aspartate must receive its amino nitrogen from glutamate via transamination, a conclusion which has been reached by others (11, 19). The fact that GOT is derepressed during the early exponential phase, along with NADP-GDH, lends further weight to this conclusion. In addition, Tempest et al. (20) showed that the concentration of glutamate in the amino acid pool of yeast is the highest of any amino acid. Brown and Johnson (4) showed that the NADP-GDH activity varies directly with the availability of ammonia and size of the amino acid pool. These facts further substantiate the premise that inorganic ammonia must be assimilated primarily through glutamate.

On examination of four strains of yeast grown on either complete or defined medium, NADP-GDH had the highest activity of any ammonia assimilation enzyme at any stage of growth (Table 1 and Fig. 2-5). Although an NADP-ADH and a glutamine synthetase were present in each strain, they displayed only minimal activity throughout growth, suggesting that they play a relatively minor role in the assimilation of inorganic nitrogen. The

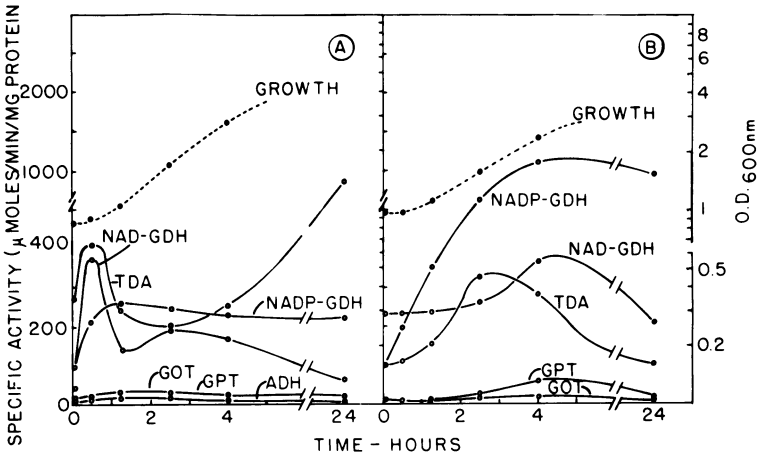

FIG. 5. *Effect of cultural conditions on the specific activity of ammonia assimilation enzymes in the GOT-deficient mutant, Saccharomyces cerevisiae X-1049-2B. The organism was transferred from (A) complete to complete and (B) complete to defined medium. Abbreviations as for Fig. 2.*

alanine dehydrogenase was found to be nicotinamide adenine dinucleotide phosphate-dependent as found in *N. crassa* (5). NADP-GDH reached maximum activity very early in the transition phase, between the lag and exponential phases of growth, at a time when the rapid assimilation of ammonia would be most critical to the growth of yeast cells. Thus the activity was greatest when the demand for synthetic activity was highest. Although most of the enzymes under investigation displayed an increase in activity at this time, NADP-GDH displayed the most marked increase. The activity was greatest when the yeasts were grown on the defined medium. These results are similar to those found in *Bacillus thuringensis* (16), where NADP-GDH activity also increased in early exponential phase. NADP-GDH appears to be the major enzyme concerned with ammonia assimilation in the four yeasts studied. In the GOT-deficient mutant, there was no detectable aspartase or glutamine synthetase. It was expected that, if aspartase were present in yeast, it should have been derepressed in this strain as a compensatory mechanism to offset the absence of the transaminase activity. Instead, methionine, threonine, aspartate, and lysine had to be supplied to support the growth of this strain, indicating the importance of the transaminase in the nitrogen economy of yeast.

The GOT, NADP-ADH, and NADP-GDH activities were derepressed and maximal when yeasts were grown on the defined medium, in which ammonia serves as the only nitrogen source, a situation in which transamination would be essential for growth if the NADP-GDH is the major route of inorganic ammonia assimilation. It appears that GOT activity is coordinately repressed and derepressed along with NADP-GDH and NADP-ADH. These enzymes must act in concert with one another to provide for maximal assimilation of inorganic nitrogen and distribution of amino nitrogen during the early period of exponential growth. During growth on a complete medium, these activities were repressed but never diminished to a point where their activities were undetectable. These findings suggest that the NADP-linked alanine dehydrogenase and glutamine synthetase must play a minor role in inorganic nitrogen assimilation in the strains of *S. cerevisiae* which were examined. GOT was highly active in all strains except the GOT-deficient mutant.

Threonine deaminase was repressed in conjunction with the NAD-GDH as was previously shown (12). Kohlaw et al. (13) also showed that glutamine synthetase was regulated in coordination with NAD-GDH. In our experience, the levels of glutamine synthetase were so low that no clear-cut relationship could be established with the other assimilation enzymes. If the cultural conditions had been identical to those used by Holzer et al. (12), perhaps the relationship would have been more evident. Likewise, although threonine deaminase followed NAD-GDH, it did not follow as strictly as Holzer had reported.

The NAD-GDH and threonine deaminase evidently serve as anabolic enzymes supplying ammonia nitrogen to the ammonia pool of yeast cells, as indicated by the fact that these enzymes are at their highest levels of activity

in cells which have been grown on the complete medium. The fact that NAD-GDH gradually increases in activity during stationary phase implies that NAD-GDH serves primarily to return amino nitrogen to the ammonia pool.

In all strains, NAD-GDH and NADP-GDH displayed the same inverse relationship of activity described by others (10, 11, 17, 18). The most rapid increase in NADP-GDH activity coincided with the most rapid decline in NAD-GDH activity. Conversely, the time of most rapid increase in NAD-GDH activity corresponded to the time of most rapid decline of NADP-GDH activity. All conditions which were found to repress the activity of one derepressed the activity of the other.

Barratt and Strickland (2) reported that NADP-GDH could reductively aminate and oxidatively deaminate several α-keto acids and α-amino acids at an efficiency of less than 5% of the activity with α-ketoglutarate or glutamate as substrates. Possibly the alanine dehydrogenase activity demonstrated in our strains is a result of this same activity on the part of NADP-GDH, especially since NADP-ADH and NADP-GDH displayed identical migration on polyacrylamide electrophoresis.

ACKNOWLEDGMENTS

This investigation was supported by National Science Foundation grant GB-8695.

K.W.T. was supported by Public Health Service Predoctoral Fellowship Fl-GM-43,286 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Ahmad, F., and A. Moat. 1966. Nicotinic acid biosynthesis in prototrophic and tryptophan auxotrophs of *Saccharomyces cerevisiae*. J. Biol. Chem. **241**:775–780.
2. Barratt, R., and W. Strickland. 1963. Purification and characterization of a TPN-specific glutamic acid dehydrogenase from *Neurospora crassa*. Arch. Biochem. Biophys. **102**:66–76.
3. Bigger-Ghering, L. 1955. Transamination reactions in *Saccharomyces fragilis*. J. Gen. Microbiol. **13**:45–55.
4. Brown, C., and B. Johnson. 1970. Influence of glucose and galactose on the physiology of *Saccharomyces cerevisiae* in continuous cultures. J. Gen. Microbiol. **64**:279–287.
5. Burk, R., and J. Pateman. 1962. Glutamic and alanine dehydrogenase determined by one gene in *Neurospora crassa*. Nature (London) **196**:450–451.
6. Ephrussi, B., H. Hottinger, and J. Tavlitski. 1949. Action de l'acriflavine sur les levures. II. Etude génétique du mutant "petite colonie". Ann. Inst. Pasteur **76**: 419–450.
7. Fincham, J., and D. Stadler. 1965. Complementation relationships of *Neurospora crassa* am mutants in relation to their formation of abnormal varieties of glutamate dehydrogenase. Genet. Res. **6**:121–129.
8. Haehn, H., and H. Leopold. 1937. Über eine Aspartasewirkung der Hefe. Biochem. Z. **292**:380–385.
9. Halpern, Y. S., and H. E. Umbarger. 1960. Conversion of ammonia to amino groups in *Escherichia coli*. J. Bacteriol. **80**:285–288.
10. Hierholzer, G., and H. Holzer. 1963. Repression of the synthesis of the DPN-dependent glutamic acid dehydrogenase in *Saccharomyces cerevisiae*, with ammonia. Biochem. Z. **339**:175–186.
11. Holzer, H., G. Hierholzer, and I. Witt. 1963. The role of glutamate dehydrogenase in the linkage and regulation of carbohydrate and nitrogen metabolism in yeast. Mech. Reg. Act. Cell. Microorganisms **124**:407–416.
12. Holzer, H., M. Boll, and C. Cennamo. 1964. The biochemistry of yeast threonine deaminase. Angew. Chem. Int. Ed. Engl. **3**:101–107.
13. Kohlaw, G., W. Dragert, and H. Holzer. 1965. Paralell-Repression der synthetase von Glutamin-Synthetase und DPN-abhanger. Glutamatdehydrogenase in Hefe. Biochem. Z. **341**:224–238.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
15. Moat, A. G., and F. Ahmad. 1965. Biosynthesis and interrelationships of amino acids in yeast. Wallerstein Lab. Commun. **28**:111–136.
16. Phibbs, P. V., Jr., and R. W. Bernlohr. 1971. Purification, properties, and regulation of glutamic dehydrogenase of *Bacillus licheniformis*. J. Bacteriol. **106**:375–385.
17. Polakis, E., and W. Bartley. 1965. Changes in the enzyme activities of *Saccharomyces cerevisiae* during aerobic growth on different carbon sources. Biochem. J. **97**:284–296.
18. Sanwal, B. 1961. Diphosphopyridine nucleotide and triphosphopyridine nucleotide linked glutamic dehydrogenases of *Fusarium*. Arch. Biochem. Biophys. **93**:377–386.
19. Sims, A., and B. Folkes. 1964. A kinetic study of the assimilation of (^{15}N)-ammonia and the synthesis of amino acids in an exponentially growing culture of *Candida utilis*. Proc. Roy. Soc. London Ser. B **159**:479–502.
20. Tempest, D. W., J. Meers, and C. Brown. 1970. Influence of environment on the content and composition of microbial free amino acid pools. J. Gen. Microbiol. **64**:171–185.
21. Ternberg, J., and F. Hershey, 1960. Colorimetric determination of blood ammonia. J. Lab. Clin. Med. **56**: 766–776.
22. Williams, V., and D. Lartigue. 1967. Quaternary structure and certain allosteric properties of aspartase. J. Biol. Chem. **242**:2973–2978.