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Regulation of Glutamate Dehydrogenases During Morphogenesis of *Schizophyllum commune*

DAVID W. DENNEN[1] AND DONALD J. NIEDERPRUEM
Department of Microbiology, Indiana University Medical Center Indianapolis, Indiana

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The specific activities of two glutamate dehydrogenases (GDH), one requiring nicotinamide adenine dinucleotide (NAD) and the other specific for nicotinamide adenine dinucleotide phosphate (NADP), varied during growth of *Schizophyllum commune* as a function of the stage of the life cycle and the exogenous nitrogen source. During basidiospore germination on either glucose-NH_3 or glucose-glutamate medium, NADP-GDH increased six- to eightfold in specific activity, whereas NAD-GDH was depressed. During dikaryotic mycelial growth on either nitrogen source, the two GDH increased in a 1:1 ratio, whereas, during homokaryotic mycelial growth on glucose-NH_3, NADP-GDH activity was depressed and NAD-GDH increased six- to eightfold. Homokaryotic mycelium cultured on glucose-glutamate medium yielded high NADP-GDH activities and normal NAD-GDH activities. Intracellular NH_3 concentration and NADP-GDH activities were inversely related during spore germination and homokaryotic mycelium growth, whereas guanosine-5'-triphosphate (GTP) and L-glutamine specifically inhibited NAD- and NADP-GDH respectively in vitro. GTP inhibition was shown in extracts from cells at all stages of the life cycle. Basidiospore germling extracts contained an NADP-GDH essentially resistant to L-glutamine inhibition.

The influence of either NH_3 or L-glutamate as sole nitrogen source on the specific activity of nicotinamide adenine dinucleotide (NAD)- and NAD phosphate (NADP)-glutamate dehydrogenases (GDH) in fungi has been called coordinate regulation (15), a term which implies the simultaneous action of these metabolites or their derivatives as inducers for one enzyme and corepressors for the other. L-Glutamate has been described as a corepressor of NADP-GDH in *Neurospora crassa* (1) and *Piricularia oryzae* (8), and NH_3 has been suggested as corepressor of NAD-GDH in *Saccharomyces cerevisiae* (7). In addition, urea, one of the products of glutamate metabolism, appears to act simultaneously to induce NAD-GDH and to repress NADP-GDH in *N. crassa* (15). The repression of NAD-GDH and the induction of NADP-GDH by NH_3 in these organisms is in agreement with their cellular function documented by studies on the *am* mutants of *N. crassa* (4). In this system, NADP-GDH is anabolic (i.e., responsible for the formation of glutamate from α-ketoglutarate and NH_3), whereas NAD-GDH is catabolic and catalyzes the reverse reaction.

[1] Present address: Eli Lilly and Co., Indianapolis, Ind.

The finding that NADP-GDH activity in *Schizophyllum commune* is depressed during growth of vegetative mycelium on glucose-NH_3, the reverse of that on glucose-glutamate medium, suggested that the GDH of this basidiomycete are regulated differently than in other fungal systems (3). Furthermore, the specific activities of these enzymes also changed with morphogenesis of this mushroom (D. W. Dennen and D. J. Niederpruem, Bacteriol. Proc., p. 58, 1966). The present report assesses the influence of NH_3, L-glutamate, and other nitrogen sources on the activities of NAD- and NADP-GDH during the development of *S. commune* through basidiospore germination, homokaryotic mycelium growth, and dikaryotization.

MATERIALS AND METHODS

Culture conditions and strains of S. commune. Homokaryotic mycelium strains of *S. commune*, 699 A41B41, 845 A51B51, M1478 A41B41 lysine$^-$, 70 A2B1 uracil$^-$, and 667 A2B2 arginine$^-$, were cultured and mated on appropriately supplemented minimal medium, and basidiospores were collected as described previously (13). A homokaryotic fruiting strain, 35hf A41B51 (Mishkin and Niederpruem, *unpublished data*), was used to compare spores with the routinely used dikaryotic fruit progeny. Initial

spore inocula and subsequent germination patterns were measured turbidimetrically as described previously (12). Culture flasks were incubated at 25 C (±0.5) in the light with aeration (180 oscillations per min) in a shaker incubator (model G27, New Brunswick Scientific Co., New Brunswick, N.J.). Spores and germlings were harvested by centrifugation of the culture mixture at 2,000 × *g*, and the culture medium was decanted. The pellet was then resuspended and recentrifuged four times with repeated washing in chilled 0.08 M phosphate buffer (*p*H 6.8). Mycelium was harvested by filtration through layered cheesecloth, washed with chilled 0.08 M phosphate buffer (*p*H 6.8), and then centrifuged three times with repeated washing. Dry-weight determinations of harvested cells were made by washing with distilled water and recentrifuging two additional times; the final cell sediment was placed in tared weighing cups at 100 C until constant weight was obtained.

Preparation of cell-free extracts and enzyme assays. The pellet of harvested cells was transferred into an ice-cold French pressure cell (Aminco, Silver Springs, Md.), and an approximately constant pressure of 10,000 psi was maintained during extraction. The extract was centrifuged at 1,800 × *g* for 10 min, intact unbroken cells and cell debris were discarded, and the supernatant fluid was centrifuged at 21,000 × *g* to remove large particulate matter. These manipulations were performed in an International model B-20 refrigerated centrifuge (International Equipment Co., Needham Heights, Mass.) equipped with a model 870 head. Further centrifugation was performed in a Spinco model L centrifuge (Beckman Instruments, Inc., Fullerton, Calif.) equipped with a type 40 head. The extract was centrifuged for 90 min at 93,000 × *g*, and the final supernatant fluid was usually clear with a lipid layer on the surface which was removed by pipetting or absorption into filter paper. This final supernatant fluid was either dialyzed, fractionated by various biochemical procedures, or assayed directly for components of low molecular weight or for enzyme content.

Glutamate dehydrogenases were assayed by measuring either the oxidation or reduction of the appropriate nicotinamide adenine dinucleotide at 340 mμ in a Zeiss model PMQII spectrophotometer with a 1-cm light path and equipped with a water-jacketed chamber at 30 C. The following reaction mixtures usually gave maximal enzyme rates, although in each assay of an unknown sample a number of substrate levels and cofactor concentrations were tested to assure complete enzyme saturation. To measure glutamate oxidation, 1.0 ml of the reaction mixture contained: enzyme preparation, 0.1 ml; potassium glutamate, 50 μmoles; NAD, 0.45 μmole, or NADP, 0.36 μmole; and tris(hydroxymethyl) aminomethane chloride (Tris chloride) buffer, 75 μmoles, previously adjusted to the proper *p*H. To measure glutamate formation, 1.0 ml of reaction mixture contained: enzyme preparation, 0.1 ml; potassium α-ketoglutarate, 5 μmoles; $(NH_4)_2SO_4$, 40 μmoles (neutralized with KOH); either reduced nicotinamide adenine dinucleotide ($NADH_2$), 0.23 μmole, or reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$), 0.18 μmole; and Tris chloride buffer, 50 μmoles. Endogenous activity was always measured in the presence of enzyme and appropriate coenzyme and in the absence of added substrate(s). The change in optical density was measured over a 3-min period every 15 sec.

Ancillary enzymes assayed at various stages of development included cytochrome oxidase, glutamine synthetase, and dehydrogenases for glucose-6-phosphate, 6-phosphogluconate, and mannitol. Cytochrome oxidase was assayed by the method described by Hackett et al. (5), glutamine synthetase by the hydroxamate method of Lipmann and Tuttle (9), and the dehydrogenases were assayed spectrophotometrically by the same procedure described for GDH. For mannitol dehydrogenase, 0.1 ml of enzyme preparation was incubated with D-mannitol, 100 μmoles; NAD, 0.45 μmole; and Tris chloride (*p*H 9.1), 80 μmoles. Dehydrogenases for glucose-6-phosphate and 6-phosphogluconate were assayed with 0.1 ml of enzyme preparation, 5 μmoles of the respective substrates, 0.36 μmole of NADP, and 70 μmoles of Tris chloride buffer (*p*H 7.3). The dehydrogenase activities were expressed in arbitrary units, and one unit was defined as that amount of enzyme which caused an optical density increase or decrease of 0.001 in 1 min at 340 mμ under optimal conditions of assay. Although GDH were assayed in both directions of equilibrium, for conformity the results reported here are expressed in units of glutamate oxidation. One unit of glutamine synthetase was defined as that amount of enzyme which caused an optical density increase of 0.001 at 540 mμ (formation of hydroxamate-$FeCl_3$ complex) under optimal conditions of assay. Specific activity was defined as the number of enzyme units per milligram of protein. For enzyme purification, anion-exchange columns were packed by gravity by use of medium-mesh diethylaminoethyl (DEAE) cellulose (0.88 meq/g; Sigma Chemical Co., St. Louis, Mo.) which had been previously washed by standard procedures (2). Glucose content in the culture medium and cell-free extract was measured with Glucostat reagents (Worthington Biochemical Corp., Freehold, N.J.), ammonia by nesslerization or the phenol-hypochlorite method (11), and total protein by the method of Lowry et al. (10) with purified bovine serum albumin as the standard. For rapid estimates of protein, the spectrophotometric method of Warburg and Christian (17) was used.

Reagents and biochemicals. Analytical-grade reagents and solvents used in these experiments were purchased from Fischer Scientific Co. (St. Louis, Mo.), carbohydrates from Pfanstiehl Laboratories (Waukegan, Ill.), biochemicals from Sigma Chemical Co. (St. Louis, Mo.), amino acids from Cyclo Chemical Corp. (Los Angeles, Calif.), and agar and prepared media were obtained from Difco Laboratories.

RESULTS

Separation and characterization of NAD- and NADP-GDH. Initially, GDH activities in crude extracts of cells at various stages of the life cycle

of *S. commune* were partially purified to establish that different protein species catalyzed glutamate oxidation via NAD and NADP. Acid precipitation of the final cell-free supernatant fluid was the quickest procedure for enzyme separation. The turbidity of the supernatant fluid produced by addition of acid suggested an isoelectric point at *p*H 4.8, and those fractions containing NAD- and NADP-GDH were at *p*H 4.8 and 3.7, respectively (Fig. 1). The possibility that NAD-GDH was not stable at low *p*H and could thus construe an apparent separation was ruled out by adjusting a fraction from *p*H 5.0 to *p*H 3.5 for an appropriate time with no loss in activity. The dialyzed cell-free supernatant fluid applied to a DEAE cellulose column and eluted with a convex gradient of phosphate-buffered KCl yielded enzyme separation as shown in Fig. 2. A combination of acid precipitation and column chromatography yielded partially purified NAD-GDH (41-fold) and NADP-GDH (18.6-fold). Attempts to concentrate eluate fractions by pervaporation or with Carbowax inactivated the enzymes, as did a second passage through the DEAE cellulose column. Additions of ethylenediaminetetraacetate

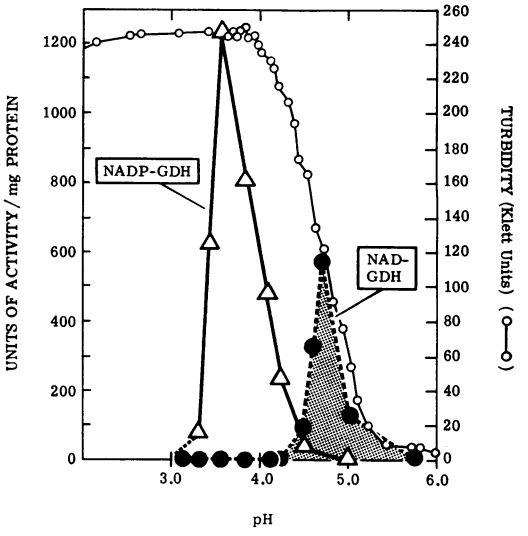

FIG. 1. *Partial purification of GDH activities of glucose-glutamate-grown Schizophyllum commune strain 699. Precipitated supernatant fluid measured in Klett units is proportional to optical density at 535 mμ; optical density = (Klett units) (500)$^{-1}$.*

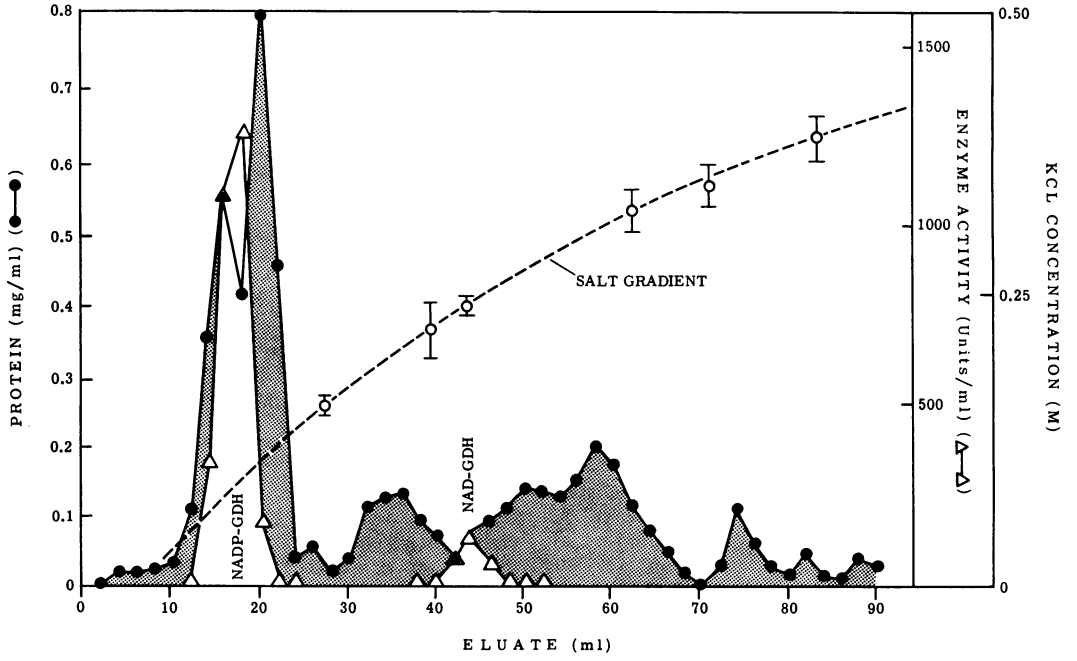

FIG. 2. *Fractionation of two GDH of Schizophyllum commune strain 699 on DEAE cellulose. A sample (5.0 ml, 11.5 mg of protein, 3,439 units of NAD-GDH, 7,843 units of NADP-GDH) of final dialyzed supernatant fluid was applied to a column (1 by 21 cm) and eluted with a constant phosphate concentration (0.01 M, pH 6.8) and increasing KCl in a convex gradient measured turbidimetrically with $AgNO_3$ in a separate experiment. The total protein recovery was 92.8%, with NAD-GDH and NADP-GDH purified 3.7- and 2.0-fold, respectively.*

(EDTA), magnesium, ethanolamine, or cysteine during purification procedures failed to stabilize the enzymes. The two enzymes differed in their *p*H optima; NAD-GDH oxidized $NADH_2$ maximally at *p*H 10.0 and reduced NAD at *p*H 8.0, whereas NADP-GDH had corresponding optima at *p*H 7.9 and *p*H 8.9. Apparent Michaelis constants (K_m) calculated from experimental data by the method of Lineweaver and Burk were, for NADP-GDH: NADP, 2.2×10^{-4} M; $NADPH_2$, 3.5×10^{-5} M; L-glutamate, 11.7×10^{-3} M; α-ketoglutarate, 3.6×10^{-3} M; and $(NH_4)_2SO_4$, 1.4×10^{-2} M. The Michaelis constants for NAD-GDH were: NAD, 1.1×10^{-3} M; $NADH_2$, 3.4×10^{-4} M; L-glutamate, 8.4×10^{-3} M; α-ketoglutarate, 1.2×10^{-3} M; and $(NH_4)_2SO_4$, 1.3×10^{-2} M. Although these values varied somewhat in extracts examined from different stages of the life cycle of *S. commune*, the degree of variation found was not considered to be significant in assigning the differences in K_m values to changes in enzyme character during morphogenesis.

Specific activity relationships of GDH and other enzymes at different morphogenetic stages. An examination of several enzyme activities at different stages of morphogenesis of *S. commune* (Table 1) suggested important roles for NADP-GDH during spore germination and for NAD-GDH during homokaryotic mycelial growth. These observed changes in GDH upon development of *S. commune* prompted an assessment of GDH activities during growth and morphogenesis with a variety of nitrogen sources, and in particular two of the GDH substrates, glutamate and NH_3. Intracellular distribution of NAD- and NADP-GDH was deduced at different morphogenetic stages of *S. commune* by an examination of the enzyme activities in differentially centrifuged supernatant fluids obtained from cells extracted in a glass homogenizer with phosphate-sucrose buffer (0.08 M phosphate, 0.25 M sucrose; *p*H 6.8). Cytochrome oxidase activity was used as a measure of particulate matter. The results revealed that, under the conditions used for extraction, the two GDH were derived essentially from the soluble cytoplasm of *S. commune*, and no changes in partition could be detected during morphogenesis. Figure 3 illustrates the disparate ratios of NAD- and NADP-GDH during spore germination on either glucose-NH_3 or glucose-glutamate minimal medium. The spores used in these studies were derived from dikaryotic fruits (e.g., 699 A41B41 × 845 A51B51) which yielded four meiotic products among the basidiospores. It was further shown that the genetically heterogeneous population was not a factor in the observed enzyme activities of basidiospore germlings, because spores derived from the homokaryotic fruiting strain 35 hf were all genetically homogeneous (i.e., A41B51) and yielded virtually identical enzyme activity changes during germination. NADP-GDH activity increased during germination on all nitrogen sources examined, including yeast extract plus peptone, L-asparagine, or L-leucine.

NAD- and NADP-GDH during homokaryotic and dikaryotic mycelial growth. Growth of several separate homokaryotic mycelia of *S. commune* (strains 845, 699, 70, 667, M1478) on glucose-NH_3 and glucose-glutamate media (appropriately supplemented) also resulted in striking changes in enzyme activities. Unlike previously described GDH activity responses for spore germination in *S. commune* or in other fungal mycelial systems, NAD-GDH increased in specific activity on glucose-NH_3 with culture age and NADP-GDH decreased, the reverse of activity on glucose-glutamate medium. It is important to note that enzyme activities in extracts of low, as compared with high, specific activity were equally stable with time during the assay. Furthermore, the ratios of the respective GDH decayed similarly upon storage. Depressed NADP-GDH and increased NAD-GDH activities during homo-

TABLE 1. *Specific activities of various enzymes during morphogenesis of Schizophyllum commune on glucose-asparagine medium*

Enzyme	Specific enzyme activity[a] (units/mg of protein)				
	Spore	Germling	Homokaryon	Dikaryon	Fruitbody
NADP-glucose-6-phosphate dehydrogenase	1,050	3,900	800	502	420
NADP-6-phosphogluconate dehydrogenase	300	400	1,550	570	400
NADP-GDH	89	703	91	317	308
NAD-mannitol dehydrogenase	200	301	1,500	267	253
NAD-GDH	106	88	698	314	298

[a] Enzyme activity measured in dialyzed final supernatant fluids.

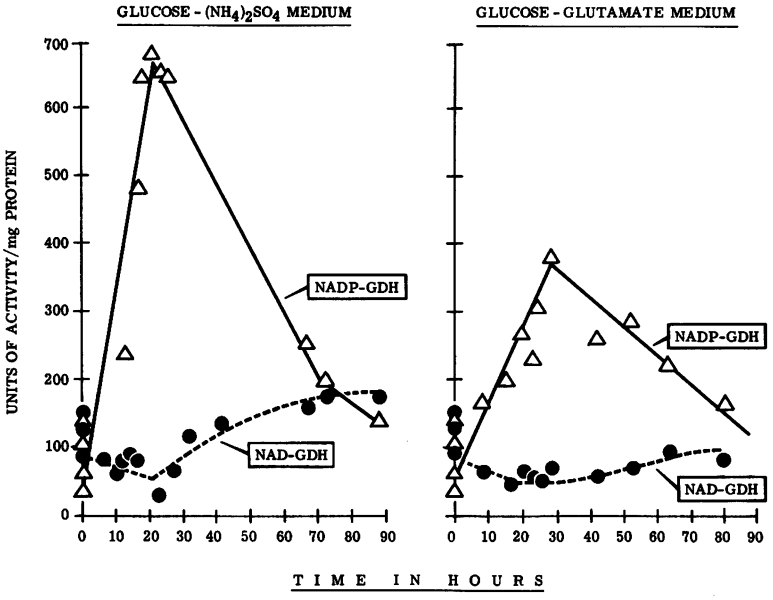

FIG. 3. *Changes in specific activity of Schizophyllum commune GDH during basidiospore germination on different culture media.*

karyotic mycelium growth were also observed when the organism was cultured on glucose-yeast extract plus peptone medium or glucose minimal medium containing NH_3, L-asparagine, or L-leucine as the sole nitrogen source. Enzyme activities of NAD- and NADP-GDH during dikaryotic mycelium growth on glucose-NH_3 medium exhibited yet another characteristic profile, again differing from either the spore germination or homokaryon systems in *S. commune*. Figure 4 shows parental homokaryons and a nutritionally forced dikaryon as a function of culture age on glucose-NH_3 medium. These individual homokaryons also showed a depressed NADP-GDH activity on glucose-NH_3 medium, although strain 677 lagged somewhat behind strain M1478 and other strains of *S. commune* in this regard. The approximate 1:1 ratio of enzyme activities during dikaryotic mycelium growth on this medium is congruent to the profile found in the same dikaryon grown on yeast extract plus peptone or glucose-glutamate media (data not shown). The nutritionally forced mating was performed to assure complete dikaryotization, but the wild-type 699 A41B41 × 845 A51B51 cross yielded equivalent results.

The possibility that NAD- and NADP-GDH specificities resided on the same enzyme in the dikaryon was not substantiated, as the typical DEAE cellulose profile was obtained with separated activities. The ratios of the two GDH specific activities could not be altered by dialysis, freezing-thawing, or the direction of equilibrium in which the enzymes were assayed. It is apparent that the two GDH activities of *S. commune* changed differently during morphogenesis of this mushroom, and were influenced by the type of exogenous nitrogen source (e.g., L-glutamate as opposed to others tested) only during homokaryotic mycelium growth.

Influence of cycloheximide on GDH activities of homokaryotic mycelium. To examine the relation of NH_3 and glutamate at this stage of development, homokaryotic mycelia were cultured for 5 days on either glucose-NH_3 or glucose-glutamate medium and then were harvested and reinoculated into the opposite medium in the presence and absence of cycloheximide (5 μg/ml) as a potential protein synthesis antagonist. In the absence of cycloheximide, the GDH specific activities showed an initial refractory phase, and after 20 hr emerged into a relationship predicted by entire growth on the second medium. The NAD-GDH activity in both cases shifted down, and therefore was not affected by the qualitative nature of the nitrogen source, but rather by the physical transfer per se. However, the NADP-GDH activity shifted down in the transfer from glutamate to ammonia and shifted up in the reverse transfer. This suggested that NADP-GDH rather than NAD-GDH activity was indeed responsive to the nitrogen source. The presence of cycloheximide

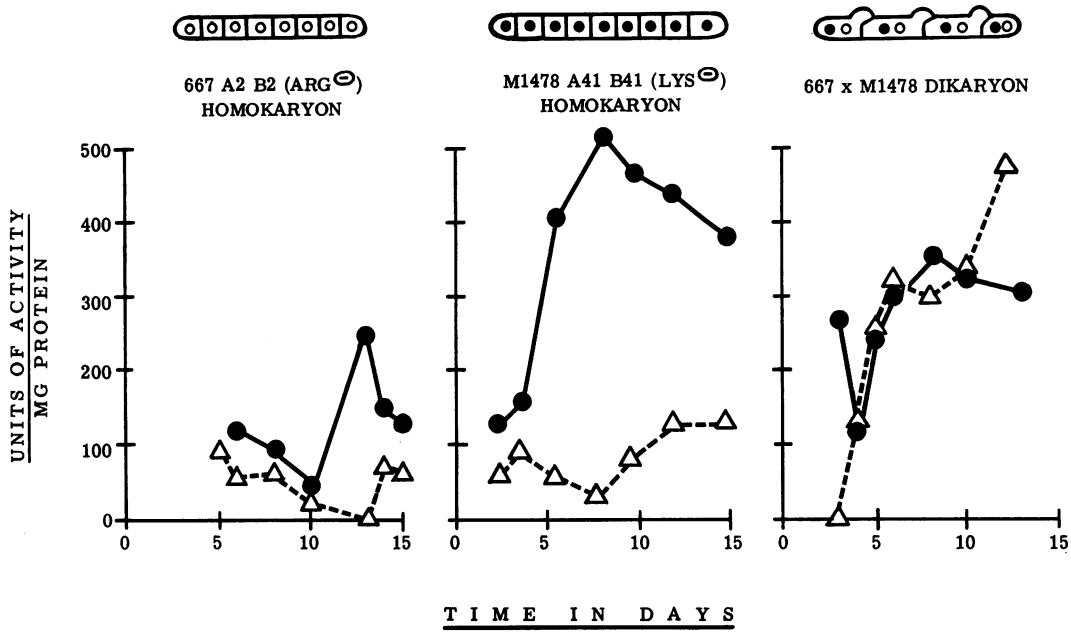

FIG. 4. *Changes in specific activity of Schizophyllum commune GDH with culture age of parent strains and their dikaryon on glucose-NH_3 medium. NAD-GDH* (●), *NADP-GDH* (△).

inhibited the increase of NADP-GDH in cultures transferred to glucose-glutamate medium while preventing its decrease in those cultures transferred to glucose-NH_3 medium. In comparison, NAD-GDH was relatively unaffected (Table 2).

The depressive influence of NH_3 on NADP-GDH activity was directly implied at the homokaryotic mycelium stage of development, but was not easily understood at either the stage of spore germination or dikaryotic mycelium growth. The interaction of two genetically dissimilar nuclei per cell in the dikaryon suggested a complicated internuclear regulation of the two GDH. It was, therefore, decided to compare the relationship between intracellular NH_3 concentration and NADP-GDH activity in the spore germination system, and also during purely vegetative homokaryotic mycelium growth. The results shown in Fig. 5 suggested that the increase in NADP-GDH on glucose-NH_3 during spore germination could be understood in terms of a low intracellular NH_3 level observed at this stage of development (i.e., insufficient corepressor is available), whereas the homokaryon had a comparatively higher internal NH_3 concentration during mycelium growth.

Effect of L-glutamine on NADP-GDH. In addition to the possible role of NH_3 in the regulation of NADP-GDH, other control mechanisms might also be operative such as transient feedback dampening of GDH activity. A number of amino acids and nucleotides, known to be related to the metabolism of L-glutamate in other systems, were examined for inhibitor properties of NAD- and NADP-GDH activity when these enzymes were extracted from different stages of the life cycle of *S. commune*. L-Glutamine showed striking inhibitor properties in vitro, and was of particular interest because it preferentially affected NADP-GDH. The oxidation of L-glutamate by NADP-GDH was inhibited by L-glutamine, whereas the enzymatic formation of glutamate from α-ketoglutarate was unaffected. In addition, the influence of L-glutamine was *p*H-dependent, with maximal inhibition occurring in alkaline solution (Fig. 6). L-Glutamine was found to be a highly specific inhibitor of NADP-GDH, and the analogues, L-asparagine and L-glutamate-γ-ethyl ester had no appreciable effect. The kinetics of L-glutamine inhibition were examined in a 17.3-fold purified NADP-GDH preparation from a glucose-glutamate grown *S. commune* strain 699 homokaryon. The results suggested a noncompetitive interaction between L-glutamine and L-glutamate for the catalytic site of NADP-GDH. The inhibitor constant (K_i) for glutamine, as evaluated graphically, was 7.5×10^{-3} M. Increased glutamine pools in the cell-free extracts could not be quantitatively demonstrated, and thus could not be collated with those stages of the

TABLE 2. *Effect of cycloheximide on transferred cultures of Schizophyllum commune strain 699 homokaryon cultured 5 days on either NH_3 or glutamate prior to transfer*

Enzyme	GDH activities[a] (units/mg of protein)					
	Glutamate control[b]	From glutamate to NH_3	From glutamate to NH_3 + cycloheximide	NH_3 control	From NH_3 to glutamate	From NH_3 to glutamate + cycloheximide
NAD-GDH.....	320 ± 16	311 ± 11	312 ± 7	537 ± 21	316 ± 18	318 ± 8
NADP-GDH.....	298 ± 19	65 ± 6	283 ± 16	91 ± 3	159 ± 5	86 ± 7

[a] Measured at 48 hr after transfer.
[b] Cultured 5 days on control medium prior to transfer.

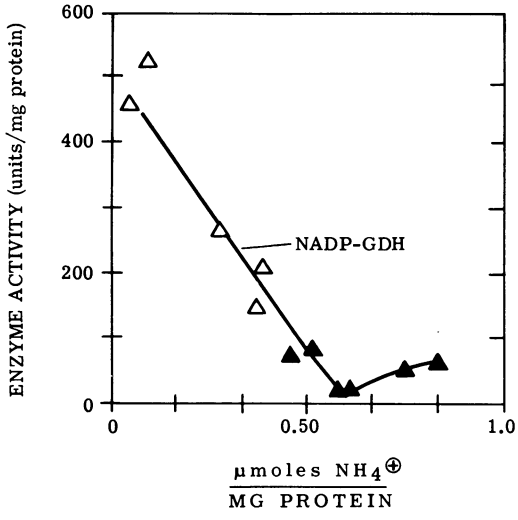

FIG. 5. *Relation between intracellular NH_3 concentration and specific activity of NADP-GDH during mycelium growth* (▲) *and during spore germination* (△).

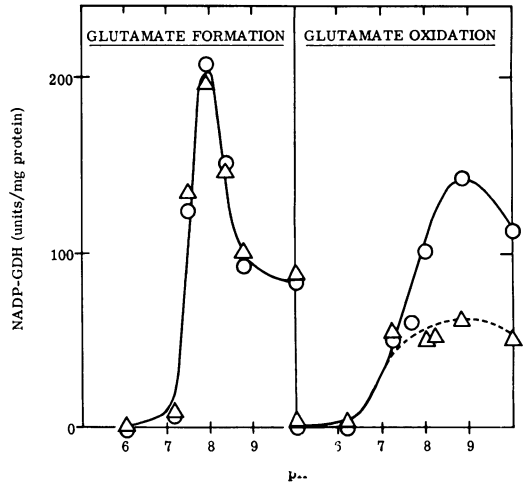

FIG. 6. *Effect of* L-*glutamine on NADP-GDH activity of Schizophyllum commune as a function of pH. Enzyme activity control* (○); *in the presence of 0.01* M L-*glutamine* (▲). *NADP-GDH purified 12.7-fold. Buffers: pH 6 to 6.5 phosphate-citrate, pH 7 to 7.5 phosphate, pH 7.9 to 10 Tris chlorides.*

life cycle of *S. commune* in which feedback inhibition could be imagined to regulate NADP-GDH.

The ancillary enzyme, glutamine synthetase, responsible for glutamine synthesis from glutamate and NH_3 was also examined in an attempt to relate its specific activity in homokaryons to the type of exogenous nitrogen source supplied. Glutamine synthetase of *S. commune* had a *p*H optimum between 6.8 and 7.6 and required adenosine triphosphate (apparent $K_m = 6.5 \times 10^{-4}$ M), glutamate (apparent $K_m = 3.1 \times 10^{-3}$ M), NH_3 (apparent $K_m = 3.1 \times 10^{-3}$ M) or NH_2OH (apparent $K_m = 2.7 \times 10^{-3}$ M), and magnesium for activity; magnesium could not be replaced by manganese. The specific activity of this enzyme was depressed on glucose-NH_3 medium and increased fourfold with culture age on glucose-glutamate medium.

Inhibition of NAD-GDH by guanosine-5′-triphosphate. Stachow and Sanwal (16) reported that NAD-GDH of *N. crassa* was inhibited by guanosine triphosphate (GTP) to the complete exclusion of NADP-GDH in this organism. The differential inhibition of the GDH of *N. crassa* offered a means of cellular control of one of the two enzymes by a nucleotide end product of a metabolic pathway leading from L-glutamate and including L-glutamine (6). Since L-glutamine appeared to be a selective inhibitor of NADP-GDH in *S. commune*, it was important to establish a role of GTP as a potential inhibitor of GDH in this basidiomycete. Subsequent experiments revealed that GTP was a specific inhibitor of *S. commune* NAD-GDH at all stages of morphogenesis, whereas it had no effect on NADP-GDH. The kinetics of GTP inhibition with *S. commune* NAD-GDH are shown in Fig. 7, and describe a

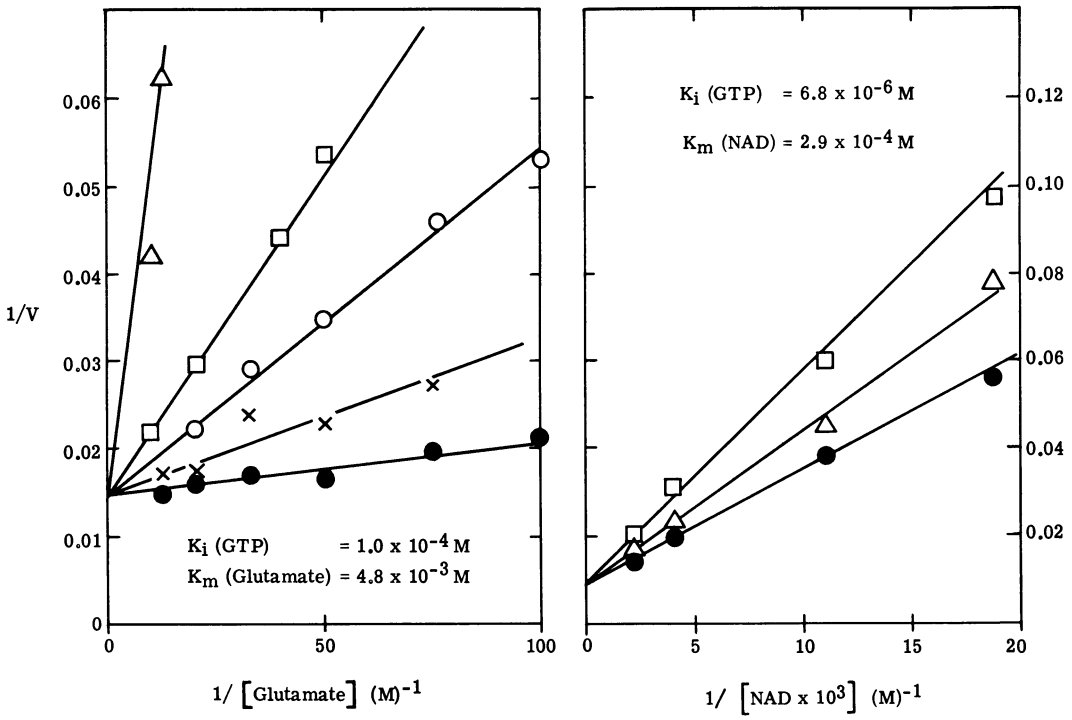

FIG. 7. *Inhibition of NAD-GDH from Schizophyllum commune glucose-NH_3-grown homokaryon by guanosine-5'-triphosphate. GTP concentrations: 0.0020 M (△); 0.0015 M (□); 0.0010 M (○); 0.0005 M (×); 0 (●). GTP concentrations: 0.001 M (□); 0.0005 M (△); 0 (●).*

competitive inhibition of this nucleotide for both NAD and L-glutamate. A comparison of the effects of L-glutamine and GTP on NAD- and NADP-GDH during development of *S. commune* is shown in Table 3; the data suggest that one intermediate (L-glutamine) and one end product (GTP) of a common metabolic pathway leading from glutamate could differentially regulate the two enzyme activities responsible for the initial synthesis of glutamate from α-ketoglutarate and NH_3. It is important to note that NADP-GDH from 20-hr basidiospore germlings of *S. commune* was essentially resistant to L-glutamine, suggesting a different form of the enzyme at this stage of development. A glutamine-resistant NADP-GDH was also shown in cell-free extracts of homokaryotic fruit basidiospore germlings (i.e., 35 hf A41B51).

DISCUSSION

The present study shows that exogenous NH_3 exerts a regulatory effect specifically on NADP-GDH and, more importantly, only at a particular stage of the developmental life cycle of *S. commune*. During basidiospore germination, significant increases in specific activity of NADP-GDH occur on either glucose-NH_3 or glucose-glutamate medium, whereas during mycelium growth this enzyme appears markedly depressed only in NH_3 medium. The depression of NADP-GDH is cycloheximide-sensitive, and therefore could imply a role of corepressor for this particular nitrogen source. Although it is appealing to consider that the basidiospore genome is unresponsive to exogenous NH_3 during early germination, we suggest that rapid turnover of ammonia at this stage of development might be a more reasonable interpretation for these findings. The role of ammonia in the regulation of NADP-GDH in *S. commune* can be contrasted with the repression of an NAD-GDH by ammonia in yeast (7). In *S. commune*, the only point in the life cycle where NAD-GDH appears to be markedly depressed is during spore germination. Whether the latter may also involve regulation of specific protein synthesis in this basidiomycete remains a matter of conjecture.

An additional possible cellular regulatory factor in *S. commune* is the susceptibility of NADP-GDH to L-glutamine during mycelium growth. Interestingly, during spore germination, sensitivity to this substance is absent and cannot be

TABLE 3. *Differential inhibition of Schizophyllum commune GDH by L-glutamine and guanosine-5'-triphosphate*

Culture	Enzyme	Purification (fold)	Per cent inhibition of enzyme activity[a]	
			L-Glutamine	GTP
Spores	NAD-GDH	7.2	0	12.0
	NADP-GDH	3.5	67.0	0
Germlings, 20 hr on glucose-NH_3	NAD-GDH	5.1	0	33.0
	NADP-GDH	17.4	0	0
Germlings, 20 hr on glucose-glutamate	NAD-GDH	0	0	38.0
	NADP-GDH	16.6	0	2.0
Homokaryon, 7.2 days on glucose-NH_3	NAD-GDH	32.0	0	50.0
	NADP-GDH	3.5	69.0	0
Homokaryon, 7.2 days on glucose-glutamate	NAD-GDH	41.0	1.0	49.0
	NADP-GDH	18.3	72.0	3.0
Dikaryon, 9.3 days on glucose-NH_3	NAD-GDH	36.9	0	52.0
	NADP-GDH	17.1	75.0	0
Dikaryon, 8.9 days on glucose-glutamate	NAD-GDH	29.7	3.0	54.0
	NADP-GDH	15.3	72.0	0

[a] GDH control activities adjusted to 40 units with inhibitor final concentrations: L-glutamine, 0.01 M; GTP, 0.001 M.

attributed to the presence of glutaminase in the germling extract. The emergence of a glutamine-resistant form of NADP-GDH during germination could allow some glutamate formed or present to undergo oxidation to α-ketoglutarate and supply the tricarboxylic acid cycle with carbon, with the subsequent formation of other amino acids through conventional transamination reactions. The evidence for this interpretation rests on the finding that L-glutamine specifically inhibits NADP-GDH in vitro only in the direction of glutamate oxidation. This type of control would not seem unreasonable during germination, since the sole source of glutamate or α-ketoglutarate must be derived from NADP-GDH, as barely detectable levels of NAD-GDH are present during this stage of development.

During purely vegetative mycelial growth of several individual strains of *S. commune*, the respective GDH activities are essentially the reverse of that of spore germination on glucose-NH_3 (e.g., low NADP-GDH and high NAD-GDH activities). Moreover, both enzymes show high specific activities in mycelial extracts after growth on glucose-glutamate medium. In the case of the former, the sole source of glutamate may reside in NAD-GDH, which always is higher in specific activity than NADP-GDH. The sensitivity of NAD-GDH to GTP could present a selective cellular control for glutamate oxidation. In the case of mycelium cultured on glucose-glutamate media, both GDH activities increase, and the dual regulation of these enzymes is inherent in glutamine and GTP sensitivities, respectively. Since glutamine inhibits NADP-GDH in its oxidation of glutamate, this enzyme presumably would be the primary enzyme for α-ketoglutarate amination. Additionally, mycelia cultured on glucose-NH_3 have low glutamine synthetase activity, whereas the reverse is true on glucose-glutamate medium. Similar repression of glutamine synthetase has been reported for yeast, with the suggestion that NH_3 replaces the requirement for glutamine in certain amino group donor reactions (H. Holzer, 6th Intern. Congr. Biochem.). The escape of glutamate into the tricarboxylic cycle via NADP-GDH may be blocked by glutamine levels corresponding to high glutamine synthetase activity on this medium, leaving NAD-GDH essentially responsible for the supply of α-ketoglutarate by glutamate oxidation. The latter could be transiently regulated by GTP, depending on the overall biosynthetic demands of the cell.

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