JOURNAL OF BACTERIOLOGY, Dec. 1980, p. 983-990
0021-9193/80/12-0983/08$02.00/0

Vol. 144, No. 3

Regulation of Glutamic Acid Decarboxylase During *Neurospora crassa* Conidial Germination

R. LYLE CHRISTENSEN AND JOSEPH C. SCHMIT*

School of Medicine and Department of Chemistry and Biochemistry, Southern Illinois University at Carbondale, Carbondale, Illinois 62901

Glutamic acid decarboxylase (GAD) from *Neurospora crassa* was assayed in dormant and germinating conidia that had been permeabilized by toluene and methanol. *N. crassa* conidia contained 10 times the GAD activity found in vegetativemycelia. During conidial germination, GAD activity rapidly decreased to low levels before germ tubes appeared. GAD activity in germinating conidia closely followed the decreasing rate of glutamic acid metabolism. Inhibiting protein synthesis partially blocked the decrease in GAD activity, but eliminating exogenous carbon sources did not alter the initial rate of decrease in this enzyme. However, when conidia were incubated for more than 3 h in distilled water, GAD activity began to increase and eventually reached levels comparable to those in dormant conidia. Either GAD was reversibly inactivated or this enzyme could be synthesized from endogenous storage compounds when conidia were incubated in distilled water. These results are consistent with the hypothesis that GAD is a developmentally regulated enzyme that is responsible for catalyzing the first step in the metabolism of the large pool of free glutamic acid during conidial germination.

Neurospora crassa conidia have many of the properties of dormant spores, including chemical and heat resistances (20). They also contain large storage pools of readily metabolizable compounds such as glutamic acid (19) and trehalose (13). As soon as conidia contact water, they lose their dormant properties and begin the early phases of germination by initiating the metabolism of these storage compounds (19). Conidia can even initiate some of the early metabolic changes that are normally associated with germination when they are exposed to 100% humidity for extended periods of time (10). An exogenous carbon source, however, is required to complete the germination phase of the asexual cycle of *N. crassa* which culminates in the formation of a germ tube (20). The enzymes that are responsible for the metabolism of these endogenous storage compounds are packaged into the conidia during conidiation (19, 20). The activity of these enzymes must be regulated in some manner to prevent the premature metabolism of these storage compounds. Understanding the mechanisms that control the activity of these enzymes is crucial for understanding the mechanisms responsible for activating dormant conidia.

In a previous study, we found that glutamic acid is the most abundant free amino acid in dormant conidia (19). This amino acid alone can account for 2.5% of the conidial dry weight. During the early phases of germination, this pool of glutamic acid is rapidly metabolized. All that is required for the initiation of glutamic acid metabolism is exposure to water. The first step in glutamic acid metabolism is thought to be its decarboxylation by glutamic acid decarboxylase (GAD) to γ-aminobutyric acid (GABA) (19). GABA is further metabolized by the enzymes of the GABA pathway and the citric acid cycle (Fig. 1). The function of this pathway could be to supply citric acid cycle intermediates and reduced coenzymes for conidial germination. This pathway was proposed because it can account for the appearance of GABA during the first few minutes of conidial germination and for the nearly stoichiometric relationship between the decrease in glutamic acid and the increase in aspartic acid pools that occur during the first 20 min of conidial germination.

The formation of GABA is one of the earliest biochemical events that occur during conidial germination (19). GABA is the only amino acid that was not found in dormant conidia but was found in germinating conidia. GABA has also been implicated in the germination of seeds (9), *Bacillus thuringiensis* endospores (1), and *Bacillus megaterium* endospores (12). Aronson et al. (1) suggested that GABA was involved in *B. thuringiensis* endospore germination. They found that GAD activity increased to a high level during sporulation. A mutant strain of *B.*

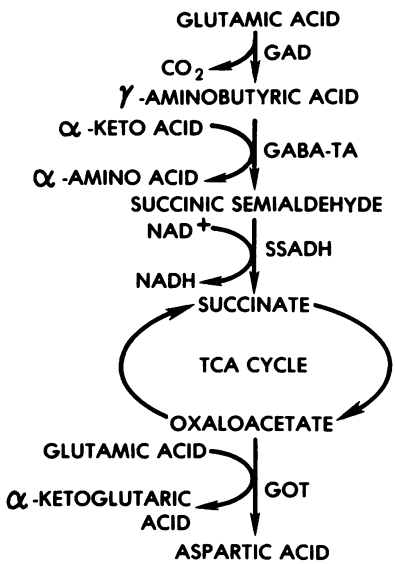

FIG. 1. *Proposed pathway for glutamic acid metabolism in N. crassa conidial germination. Abbreviations: GAD, glutamic acid decarboxylase (EC 4.1.1.15); GABA-TA, γ-aminobutyric acid transaminase (EC 2.6.1.19); SSADH, succinic semialdehyde dehydrogenase (EC 1.2.1.16); TCA cycle, tricarboxylic acid cycle; and GOT, glutamate-oxaloacetate transaminase (EC 2.6.1.1).*

megaterium that requires GABA for germination has been isolated (11). This mutant strain had low levels of GAD activity in its endospores which prevented the degradation of its endogenous glutamic acid pool (11). The germination of these endospores required exogenous GABA. Thus, the metabolism of glutamic acid by GAD may be a critical event required for the germination of dormant cells from a variety of organisms.

The rate of glutamic acid metabolism during conidial germination could be controlled by regulating the activity of GAD, the first enzyme in this pathway (Fig. 1). GAD has not been studied previously in *N. crassa*. We have begun to characterize the catalytic and regulatory properties of GAD as a function of the asexual development cycle of *N. crassa*. In a preliminary study, we found that conidia contain high levels of GAD activity (5). The following work describes some of the properties of GAD and shows that this enzyme is regulated during germination. The unique changes in GAD activity that occur during the asexual development cycle of *N. crassa* are consistent with the hypothesis that this enzyme is responsible for the metabolism of glutamic acid during conidial germination.

MATERIALS AND METHODS

Strain and chemicals. The *nada* strain of *N. crassa* (FGSC 2688) was used in these studies. This strain lacks the extracellular enzyme $NAD(P)^+$ glycohydrolase (18) and can be obtained from the Fungal Genetics Stock Center, Humboldt State College, Arcata, Calif. The radioactive amino acids were obtained from New England Nuclear Corp., Boston, Mass. All other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo., except where noted.

Harvest and germination of conidia. The conidia were obtained from cultures grown on agar slants at 24°C in test tubes (18 by 150 mm) on a complete medium (21) containing 1% Casamino Acids, 0.5% yeast extract, 2% sucrose, 1% glycerol, and Vogel salts (25). Dry-harvested conidia were obtained by inverting the culture tube and tapping the conidia into a preweighted tube (19).

The dry-harvested conidia were used to inoculate either liquid medium containing 2% glucose and Vogel salts (25) or distilled water. The final concentration of the inoculum was 1.5 mg of freshly harvested conidia per ml of medium. These cultures were incubated at 24°C on a rotary shaker at 150 rpm. Samples containing 5 ml of germinating conidia were mixed with 185 nmol of cycloheximide and placed on ice. The samples were centrifuged at 2,000 rpm for 6 min at 4°C, the supernatant was removed, and the conidia were permeabilized.

Permeabilization of conidia. The conidia were permeabilized by a modification of the procedure of Basabe et al. (2). Five milliliters of phosphate-EDTA buffer (20 mM potassium phosphate, 10 mM EDTA, pH 6.8) was added to the conidial pellet. Immediately, 300 μl of a 1:4 mixture of toluene and methanol was added. The solution was blended in a Vortex mixer for 2 min and centrifuged for 6 min at 2,000 rpm. Dry-harvested conidia were suspended directly into phosphate-EDTA buffer containing toluene and methanol. After the supernatant was removed, the conidia were washed twice with 6 ml of phosphate-EDTA buffer. The washed, permeabilized conidia were suspended in 1 ml of 100 mM Tris buffer, pH 7.1. The permeabilized conidia were either assayed immediately or stored at −80°C.

GAD assay. GAD activity was assayed by measuring the amount of $^{14}CO_2$ released from DL-[1-^{14}C]glutamic acid. The GAD assay was adapted from that of Schrier et al. (22). The reaction vessel was a test tube (10 by 37 mm). The test tube was inserted into a flanged rubber stopper (Arthur H. Thomas Co., Philadelphia, Pa.; lot 1781-E90). The whole assembly was inserted into the top of a scintillation vial (inside diameter, 15 mm) and sealed with Parafilm. The scintillation vial contained a scintillation pad (Arthur H. Thomas Co.; lot 4752-N10) that was impregnated with 35 μl of 1.0 M methylbenzethonium hydroxide. The standard reaction mixture contained 45 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] at pH 5.5, 45 mM histidine at pH 5.5, 0.9 mM pyridoxal-5′-phosphate, 0.9 mM EDTA, 0.9 mM mercaptoethanol, and up to 185 μg of permeabilized conidia. The reaction was started by adding L-glutamic acid, pH 5.7. The

final glutamic acid concentration was 27 mM, with 1.1 μCi of DL-[1-^{14}C]glutamic acid per ml. The total reaction volume was 110 μl. After 30 min of incubation at 24°C, the reaction was terminated by adding 200 μl of 8 N H_2SO_4 through the rubber stopper with a syringe. The test tube was gently released to the bottom of the vial with the syringe needle. The $^{14}CO_2$ produced by GAD was absorbed by the methylbenzethonium hydroxide for at least 1 h. The test tube was removed, and 5 ml of a scintillation cocktail was added. One liter of scintillation cocktail was made by mixing 656 ml of toluene, 298 ml of methanol, and 46 ml of Liquifluor (New England Nuclear Corp.). The radioactivity was measured on a Beckman scintillation counter. The activity of GAD was measured as the nanomoles of CO_2 released per minute. All assays were done in triplicate and usually varied less than ±10%. Assays without enzyme and without glutamic acid were done as controls for each experiment, and background levels of $^{14}CO_2$ were obtained.

Protein assay. The protein concentration was measured directly in the samples of permeabilized conidia (6). The procedure was based on the "Bio-Rad Protein Assay" (Bio-Rad Laboratories, Richmond, Calif., bulletin 1060, February 1979). The permeabilized conidia were prepared for the protein assay by blending 300 μl of sample (5 to 10 mg of permeabilized conidia) with 300 μg of acid-washed sand in a Vortex mixer for 2 min. After the sand settled, the supernatant was removed and stored at 4°C. The protein content of the supernatant was determined by mixing 20 μl of the ground cells with 1 ml of dye. After 10 min, followed by gentle mixing, the absorbance was measured at 595 nm. A protein standard curve was prepared with bovine immunoglobulin G for each assay. The protein content of the sample was determined by comparison with the standard curve. The assay was very reproducible and was linear from 4 to 28 μg of protein per assay.

RESULTS

Properties of GAD. High levels of GAD activity were detected in dry-harvested conidia that had been permeabilized by toluene and methanol (Table 1). Very little GAD activity was found in cell-free extracts, and none was released into the supernatant when the conidia were permeabilized (Table 1). Pyridoxal-5'-phosphate was required for maximal activity (Table 1). Little activity was detected when glutamine or ornithine was used as an alternate substrate. The specific activity of GAD in dry-harvested conidia averaged 152 ± 50 nmol of CO_2 min^{-1} mg of protein^{-1}. Very similar specific activities for GAD were obtained with an assay that directly measured GABA production rather than CO_2 evolution (5).

Under standard assay conditions, the production of CO_2 increased linearly with respect to time and to the milligrams of permeabilized conidia that were added to the assay mixture (Fig. 2). The linearity of these results indicates that no product inhibition of GAD occurred during the period of the reaction and that the GAD activity was stable in permeabilized conidia. Permeabilized conidia could also be stored for 2 months at −80°C with a loss of GAD activity of less than 20%. Further, endogenous

TABLE 1. *GAD activity in N. crassa conidia*[a]

Source of enzyme	Substrate[b]	Activity (%)
Permeabilized conidia	Glutamate	100
	Glutamate (no pyridoxal-5'-phosphate)	66
	Glutamine	3
	Ornithine	4
Supernatant from permeabilized conidia[c]	Glutamate	0
Cell-free extract[d]	Glutamate	9

[a] Dry-harvested conidia were obtained as described in the text.

[b] The assay mixtures contained 26 mM L-glutamate, L-glutamine, or L-ornithine, with 0.23 μCi of DL-[1-^{14}C]glutamate, L-[*U*-^{14}C]glutamine, or DL-[1-^{14}C]ornithine per assay, respectively. The substrates were adjusted to pH 5.7.

[c] This sample was the supernatant from the toluene-methanol permeabilization of dry-harvested conidia.

[d] Conidia were ground with sand at 4°C in 100 mM potassium phosphate (pH 6.8), and the cellular debris was removed by centrifugation at 2,000 rpm for 6 min.

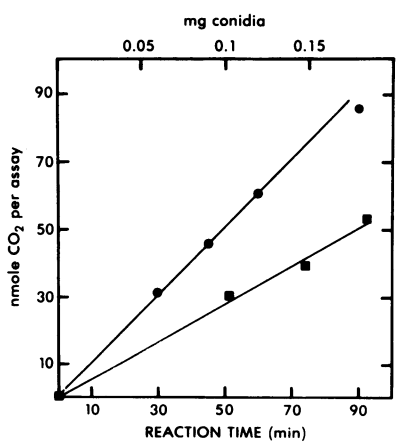

FIG. 2. *Linearity of the GAD assay. Dry-harvested conidia were permeabilized as described in the text. GAD activity was determined by measuring the CO_2 released from glutamic acid. Symbols: ●, reaction time with 0.11 mg of conidia per assay; ■, mass of permeabilized conidia assayed for 30 min.*

small molecules could not have affected the GAD activity in our assay since most of the free amino acids and other soluble small molecules were released from the conidia by the permeabilization procedure. In a control experiment, the free amino acids were extracted from conidia after the toluene-methanol treatment with boiling 80% ethanol (19) and were measured with a Beckman 119CL amino acid analyzer. The analyzer data showed that permeabilized conidia had lost more than 92% of their endogenous free amino acids and that 94% of the glutamic acid pool had been solubilized. No inhibitors were detected when dormant and germinating conidia were assayed together. These results indicated that the observed GAD activity was representative of the endogenous activity of this enzyme.

The pH optimum for GAD activity was between pH 5 and 6 when a histidine-PIPES buffer was used. In addition to pH, GAD activity was also dependent on the buffer used. Both histidine and PIPES buffers separately gave much higher activities at pH 6 than were obtained when a potassium malonate buffer was used. Equal molar mixtures of histidine and PIPES gave an activity 10 to 20% higher than did either of these buffers alone. In the combined histidine-PIPES buffer, GAD activity increased slightly from pH 6 to 5. At pH 5, the buffer components began to precipitate. Therefore, all other GAD assays reported in this paper were done at pH 5.5 in the combined histidine-PIPES buffer.

GAD activity in permeabilized conidia followed Michaelis-Menten kinetics (Fig. 3). The apparent K_m for glutamate was 3.2 mM. A glutamate concentration of 27 mM, which was 87% of V_{max}, was used to measure GAD activity in the standard assay system. GABA was found to be a competitive inhibitor of GAD activity in permeabilized conidia. The K_i for GABA was 70 mM, as determined by replotting the slopes of the curves in Fig. 3 against the GABA concentration.

Protein content of germinating conidia. One potential problem with measuring the specific activity of GAD during conidial germination could be variations in protein extraction (20). To overcome this difficulty, the protein content was assayed in the same preparation of permeabilized cells that was used for the enzyme assay. This procedure also eliminated the sampling error caused by the tendency of germinating conidia to clump together. The protein content began to increase very soon after the conidia were suspended in minimal glucose medium (Fig. 4). After 1 h, both the protein content and the total dry weight of these cultures increased, with doubling times of 2.5 h (Fig. 4). The protein

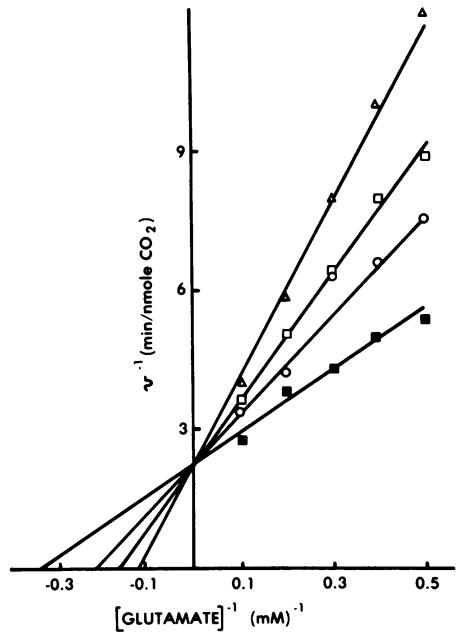

FIG. 3. *Effect of glutamic acid and GABA concentrations on GAD activity. GAD was measured in permeabilized dry-harvested conidia as described in the text. The Lineweaver-Burk plots were derived from assays of GAD activity at various glutamic acid concentrations in the presence of 0 (■), 35 (○), 75 (□), and 150 (△) mM GABA.*

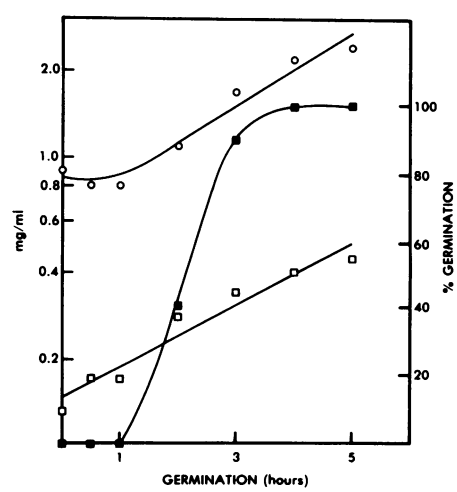

FIG. 4. *Germ tube formation, protein synthesis, and dry weight increase during conidial germination. Conidia were considered germinated when their germ tubes were one-half the diameter of the conidium (19). Protein was assayed as described in the text. Dry weight was measured after drying conidia harvested on preweighed filters at 90°C for 24 h. Symbols: ○, dry weight; ■, percent germination; □, protein concentration.*

content remained constant when conidia were incubated in distilled water and when cycloheximide was added to the growth medium (6).

Inactivation of GAD during conidial germination. The specific activity of GAD decreased rapidly during the early phases of conidial germination (Table 2 and Fig. 5). The half-life of the GAD activity was 28 min. After 3 h, most of the conidia had germinated (Fig. 4), and the specific activity of GAD had decreased to 12% of its initial activity (Fig. 5). The specific activity of GAD remained at this low level for at least another 8 h during vegetative growth. None of the GAD activity was released into the medium during germination.

The specific activities of three other enzymes that could metabolize glutamic acid changed differently than did that of GAD during conidial germination (Table 2). Dormant conidia contained moderate levels of NAD^+ and $NADP^+$ glutamate dehydrogenase [GDH(NAD^+) and GDH($NADP^+$), respectively] activities and high levels of glutamate-oxaloacetate transaminase (GOT) activity. The specific activity of GDH(NAD^+) decreased during germination but not to the extent that GAD decreased. In contrast, the specific activity of GDH($NADP^+$) increased threefold in this experiment. The specific activity of GOT decreased and then increased slightly during the early stages of conidial germination. All of these enzyme activities exhibited trends different from GAD activity,

TABLE 2. *Changes in activity of enzymes which could potentially utilize glutamate during N. crassa conidial germination*[a]

Enzyme[b]	Relative enzyme activity (%)			
	T_0-dry[c]	$T_{0.5}$	T_1	T_4
GAD	100[d]	56	38	16
GDH(NAD^+)	100	60	63	47
GDH($NADP^+$)	100	180	240	300
GOT	100	76	110	63

[a] All of the enzymes were assayed in the same samples derived from a single preparation of conidia germinated in minimal medium as described in the text.

[b] Permeabilized conidia were assayed for GAD activity and protein content as described in the text. GDH(NAD^+), GDH($NADP^+$), and GOT were assayed by the procedures of Mazon (16) and Kachmar and Moss (14), respectively. All enzyme assays were done at 23°C.

[c] The time of growth (T) was measured in hours.

[d] The percent relative activities were based on the specific activities of permeabilized dry-harvested conidia. The specific activities of the 0-dry samples were as follows: GAD, 63 nmol of CO_2 min^{-1} mg of $protein^{-1}$; GDH(NAD^+), GDH($NADP^+$), and GOT, 32, 16, and 450 nmol of NAD(P)H min^{-1} mg of $protein^{-1}$, respectively.

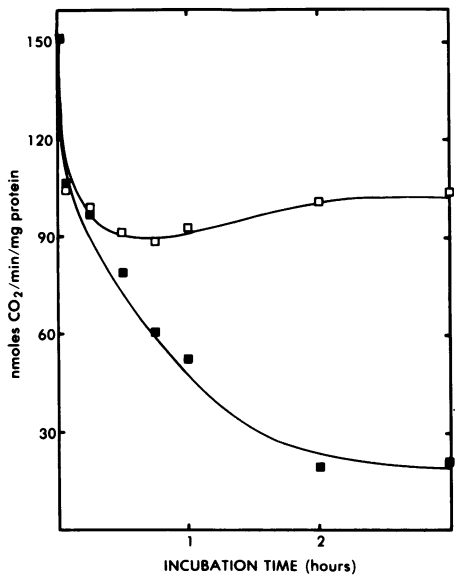

FIG. 5. *Changes in GAD activity during conidial germination. Dry-harvested conidia were used to inoculate cultures containing minimal glucose medium with* (□) *and without* (■) *36 μM cycloheximide. The cultures were incubated with shaking at 150 rpm and 24°C, and samples were removed at the times indicated. The samples were permeabilized and stored at −80°C until assayed for GAD activity and protein.*

which decreased to 16% of its initial level during this experiment.

The decrease in the specific activity of GAD was partially prevented by adding cycloheximide to the growth medium before inoculating with conidia (Fig. 5). The protein content of the conidia remained constant and no germ tubes were formed in the presence of cycloheximide. The GAD activity decreased 30 to 40% during the first few minutes of conidial exposure to cycloheximide-containing medium; however, further decreases in GAD activity were blocked (Fig. 5). These results suggest that either some inactivation of GAD occurred before cycloheximide penetrated the conidia or the very initial inactivation of GAD was not dependent on protein synthesis.

The specific activity of GAD decreased at nearly the same rates when conidia were incubated in distilled water or in minimal glucose medium (Fig. 6A). GAD decreased even though there were no visible signs of germination, and the net protein content remained constant. These results indicate that an exogenous carbon source was not required for the inactivation of GAD.

Protein synthesis was apparently necessary for the complete inactivation of GAD in distilled water, even though the net protein content of

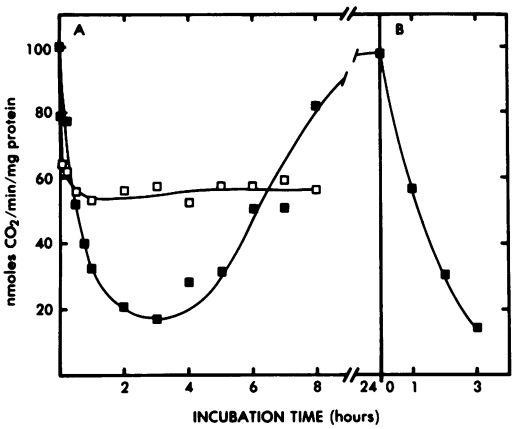

FIG. 6. *Changes in GAD activity in distilled water. (A) Dry-harvested conidia were used to inoculate distilled water with (□) and without (■) 36 μM cycloheximide. The cultures were incubated at 24°C with shaking at 150 rpm, and samples were removed at the times indicated. The samples were permeabilized and stored at −80°C until assayed for GAD activity and protein. (B) At 24 h, a portion of the culture of conidia that had been incubating in distilled water was harvested on a sterile filter and used to inoculate a flask containing minimal glucose medium. All other culture conditions were the same as for (A).*

conidia remained constant. The addition of cycloheximide to the distilled water inhibited 70% of the inactivation of GAD (Fig. 6A). The rapid initial drop in GAD activity still occurred, just as it did when conidia were incubated in minimal glucose medium with cycloheximide (Fig. 5).

The specific activity of GAD began to increase after the conidia had been incubated in distilled water for several hours and eventually reached a level comparable to that in dormant conidia (Fig. 6A). Cycloheximide added at 2 to 3 h after incubation in distilled water had little effect on the subsequent increase in GAD activity. When the conidia incubated in water were transferred to minimal glucose medium after 24 h, GAD activity decreased rapidly (Fig. 6B), just as it did when the conidia were first suspended in either distilled water or minimal glucose medium. In this experiment, the protein content began to increase at normal rates, and germ tubes began to appear within 3 h after the conidia were transferred to minimal glucose medium.

DISCUSSION

N. crassa conidia contain 5 to 10 times the GAD activity of vegetative mycelia. This enzyme is stored in conidia during conidiation, apparently for use during germination. The substrate for GAD, glutamic acid, is also stored at high levels in conidia (19). Glutamic acid is the predominant free amino acid in conidia and alone can account for up to 2.5% of the conidial dry weight (19). During germination, glutamic acid is metabolized and GABA, the product of GAD, is formed. If GAD activity remained at the level of dormant conidia, it would be sufficient to degrade all of the stored glutamic acid within 8 min. The observation that conidia contained high levels of GAD activity is consistent with the hypothesis that this enzyme plays a key role in metabolizing glutamic acid during the early phases of conidial germination (20).

GAD is one of only three intracellular enzymes that has a specific activity higher in dormant conidia than in mycelia. Only malate synthase (7) and succinic semialdehyde dehydrogenase (unpublished data) are present at levels higher in conidia than in mycelia. This second enzyme is also part of the proposed pathway for glutamic acid metabolism during germination (Fig. 1). All of the other enzymes that are stored at high levels in conidia are extracellular enzymes (20). Several experiments support the conclusion that GAD is an intracellular enzyme. GAD activity could not be washed from the surface of the conidia, and none of the activity was released into the medium during germination. Also, GAD activity could not be detected until after the conidia had been permeabilized with toluene and methanol.

The assay of GAD in a permeabilized conidial sample reflected the true enzyme activity present in that sample. Amino acid analysis of whole conidia and post-permeabilized conidia indicated that the substrate for GAD and other amino acids had been removed by the permeabilization process. The treated conidia appeared to be freely permeable to glutamate and GABA. Other low-molecular-weight molecules that might alter GAD activity would also be eliminated by the permeabilization procedure. The enzyme assay was linear over a wide range of substrate concentrations, indicating that the remaining endogenous glutamic acid pool was very small and did not interfere with the measuring of GAD activity by following CO_2 evolution. In a previous study (5), we showed that GAD activity decreased during the early stages of conidial germination by using an assay that directly measured GABA production.

The specific activity of GAD decreases rapidly during the early phases of conidial germination. The mechanism for the inactivation of GAD may be specific for this enzyme. Three other enzymes, GDH(NAD^+), GDH($NADP^+$), and GOT, which utilize glutamate as a substrate did not follow the same changes in specific activity as did GAD during conidial germination (Table 2). GDH(NAD^+) activity decreased but not as much as did GAD activity, while GDH($NADP^+$)

activity increased threefold over the same 4 h. These results were similar to those reported by others (15, 16, 24). GOT activity was high in conidia and remained relatively high during germination. These results indicate that the change in GAD activity that occurs during germination was not caused by the permeabilization procedures since some activities of the enzymes were increased whereas others were decreased, even though they were assayed in the same preparation of germinating conidia

The rate that GAD activity decreased during conidial germination correlated very well with the rate of glutamic acid degradation. During the first 30 min of germination, GAD activity decreased to 50% of its initial level and about half of the stored glutamic acid was metabolized (19). Both the glutamic acid pool and the activity of GAD approached their minimum levels after 1 h. Thus, as GAD is inactivated, the rate of glutamic acid metabolism is reduced.

The inactivation of GAD is apparently dependent on protein synthesis. Cycloheximide inhibited 60 to 70% of the inactivation of GAD. The inability of cycloheximide to block the total decrease in GAD activity was probably due to the inability of cycloheximide to rapidly penetrate the dormant conidia. Conidia have a hydrophobic surface layer that is lost when they are suspended in water or growth medium (3, 8, 23). This hydrophobic surface layer could interfere with the transport of cycloheximide. Since protein synthesis is initiated very rapidly during conidial germination (4, 17), some protein synthesis-dependent inactivation of GAD could have occurred before cycloheximide was able to block protein synthesis.

The inactivation of GAD does not require exogenous carbon sources. When conidia were incubated in distilled water, GAD activity decreased to low levels and remained low for several hours. The initial rate of GAD inactivation in water was very similar to the rate of inactivation in minimal glucose medium. Cycloheximide again inhibited 60 to 70% of the inactivation of GAD. These results indicate that, even in the absence of exogenous carbon sources, protein synthesis is necessary for the inactivation of GAD. Apparently, the metabolism of endogenous storage compounds can supply the energy and precursors necessary for protein synthesis.

GAD activity begins to increase during conidial incubation in distilled water after 3 to 8 h, even though no net protein synthesis or germ tube formation occurs (Fig. 6). Thus, either GAD can be synthesized and accumulated under starvation conditions or the mechanism responsible for inactivating GAD is readily reversible. The observation that cycloheximide did not prevent the increase in GAD activity that occurred in distilled water could support the hypothesis that GAD inactivation is reversible. However, another possible explanation is that the cycloheximide could no longer penetrate the cells, perhaps due to a change in the properties of the conidia during starvation. The increase in GAD activity in distilled water indicates that the conidia can reestablish the enzymatic systems associated with dormancy in preparation for a time when environmental conditions are more favorable for completing germination.

Both glutamic acid and the degradative enzyme GAD are stored at high levels in dormant conidia. A regulatory mechanism must exist that prevents GAD from degrading glutamic acid during conidiation. This regulatory mechanism is terminated as soon as the conidia are exposed to water. Exposure to water appears to be the event that initiates glutamic acid degradation as well as other developmental changes that occur during the early phases of conidial germination (20). This regulatory mechanism must be quite efficient since GABA cannot be detected in dormant conidia, but it is found in conidia that have been briefly exposed to water (19).

A second regulatory mechanism that specifically inactivates GAD appears to be responsible for controlling the rate of glutamic acid metabolism during germination. There is a good correlation between the level of GAD activity and the rate of glutamic acid degradation in germinating conidia. As GAD is inactivated, the rate of glutamic acid metabolism is decreased. Future experiments will be designed to obtain more information about both of these regulatory mechanisms.

ACKNOWLEDGMENTS

This research was supported in part by National Science Foundation research grant PCM 77-12551 and equipment grant PCM 77-17808.

The technical assistance of Dan Wolens and the amino acid analyses by Pat Tindal are gratefully acknowledged.

LITERATURE CITED

1. **Aronson, J. N., J. F. Doerner, E. W. Akers, D. P. Borris, and M. Mani.** 1975. γ-Aminobutyric acid pathway of glutamate metabolism by *Bacillus thuringiensis*, p. 404–410. *In* P. Gerhardt, R. N. Costilow, and H. L. Sadoff (ed.), Spores VI. American Society for Microbiology, Washington, D.C.
2. **Basabe, J. R., C. A. Lee, and R. L. Weiss.** 1979. Enzyme assays using permeabilized cells of *Neurospora*. Anal. Biochem. **92**:356–360.
3. **Beever, R. E., R. J. Redgwell, and G. P. Dempsey.** 1979. Purification and chemical characterization of the rodlet layer of *Neurospora crassa* conidia. J. Bacteriol. **140**:1063–1070.
4. **Bhagwat, A. S., and P. R. Mahadevan.** 1970. Conserved mRNA from the conidia of *Neurospora crassa*. Mol. Gen. Genet. **109**:142–151.
5. **Christensen, R. L., and J. C. Schmit.** 1979. "In situ"

changes in enzyme activity during *Neurospora* conidial germination. Neurospora Newslett. **26**:13.

6. **Christensen, R. L., D. Wolens, and J. C. Schmit.** 1980. Improved techniques for assaying protein concentration in germinating *Neurospora* conidia. Neurospora Newslett. **27**:28.
7. **Combepine, G., and G. Turian.** 1970. Activities de quelques enzymes associes á la conidiogenese du *Neurospora crassa*. Arch. Mikrobiol. **72**:36–47.
8. **Dempsey, G. P., and R. E. Beever.** 1979. Electron microscopy of the rodlet layer of *Neurospora crassa* conidia. J. Bacteriol. **140**:1050–1062.
9. **Duffus, C. M., J. H. Duffus, and J. C. Slaughter.** 1972. Glutamate decarboxylase in barley aleurone and its relationship to α-amylase development during germination. Experientia **28**:635–636.
10. **Fahey, R. C., S. D. Mikolajczyk, and S. Brody.** 1978. Correlation of enzymatic activity and thermal resistance with hydration state in ungerminated *Neurospora* conidia. J. Bacteriol. **135**:868–875.
11. **Foerster, H. F.** 1971. γ-Aminobutyric acid as a required germinant for mutant spores of *Bacillus megaterium*. J. Bacteriol. **108**:817–823.
12. **Foerster, H. F.** 1972. Spore pool glutamic acid as a metabolite in germination. J. Bacteriol. **111**:437–442.
13. **Hanks, D. L., and A. S. Sussman.** 1969. The relationship between growth, conidiation and trehalase activity in *Neurospora crassa*. Am. J. Bot. **56**:1152–1159.
14. **Kachmar, J. F., and D. W. Moss.** 1976. Enzymes, p. 677–680. *In* N. W. Tietz (ed.), Fundamentals of clinical chemistry, 2nd ed. The W. B. Saunders Co., Philadelphia.
15. **Loo, M.** 1976. Some required events in conidial germination of *Neurospora crassa*. Dev. Biol. **54**:201–213.
16. **Mazon, M. J.** 1978. Effect of glucose starvation on the nicotinamide adenine dinucleotide phosphate-dependent glutamate dehydrogenase of yeast. J. Bacteriol. **133**: 780–785.
17. **Mirkes, P. E.** 1974. Polysomes, ribonucleic acid, and protein synthesis during germination of *Neurospora crassa* conidia. J. Bacteriol. **117**:196–202.
18. **Nelson, R. E., C. P. Selitrennikoff, and R. W. Siegel.** 1976. Developmental regulation of nicotinamide adenine dinucleotide (phosphate) glycohydrolase in *Neurospora crassa*. Dev. Biol. **50**:122–133.
19. **Schmit, J. C., and S. Brody.** 1975. *Neurospora crassa* conidial germination: role of endogenous amino acid pools. J. Bacteriol. **124**:232–242.
20. **Schmit, J. C., and S. Brody.** 1976. Biochemical genetics of *Neurospora crassa* conidial germination. Bacteriol. Rev. **40**:1–41.
21. **Schmit, J. C., C. M. Edson, and S. Brody.** 1975. Changes in glucosamine and galactosamine levels during conidial germination in *Neurospora crassa*. J. Bacteriol. **122**:1062–1070.
22. **Schrier, B. K., S. H. Wilson, and M. Nirenberg.** 1974. Glutamate decarboxylase (EC 4.1.1.15) assay. Methods Enzymol. **32**:779–781.
23. **Turian, G., and D. E. Bianchi.** 1972. Conidiation in *Neurospora*. Bot. Rev. **38**:119–154.
24. **Tuveson, R. W., D. J. West, and R. W. Barratt.** 1967. Glutamic acid dehydrogenases in quiescent and germinating conidia of *Neurospora crassa*. J. Gen. Microbiol. **48**:235–248.
25. **Vogel, H. J.** 1964. Distribution of lysine pathways among fungi: evolutionary implications. Am. Nat. **98**:435–446.