APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1993, p. 4317–4322
0099-2240/93/124317-06$02.00/0

Vol. 59, No. 12

Glutamine Involvement in Nitrogen Control of Gibberellic Acid Production in *Gibberella fujikuroi*

GASTÓN A. MUÑOZ AND EDUARDO AGOSIN*

Department of Chemical Engineering, Faculty of Engineering, Catholic University of Chile, P.O. Box 6177, Santiago, Chile

Received 10 June 1993/Accepted 18 September 1993

When the fungus *Gibberella fujikuroi* ATCC 12616 was grown in fermentor cultures, both intracellular kaurene biosynthetic activities and extracellular GA_3 accumulation reached high levels when exogenous nitrogen was depleted in the culture. Similar patterns were exhibited by several nonrelated enzymatic activities, such as formamidase and urease, suggesting that all are subject to nitrogen regulation. The behavior of the enzymes involved in nitrogen assimilation (glutamine synthetase, glutamate dehydrogenase, and glutamate synthase) during fungal growth in different nitrogen sources suggests that glutamine is the final product of nitrogen assimilation in *G. fujikuroi*. When ammonium or glutamine was added to hormone-producing cultures, extracellular GA_3 did not accumulate. However, when the conversion of ammonium into glutamine was inhibited by L-methionine-DL-sulfoximine, only glutamine maintained this effect. These results suggest that glutamine may well be the metabolite effector in nitrogen repression of GA_3 synthesis, as well as in other nonrelated enzymatic activities in *G. fujikuroi*.

Gibberellic acid (GA_3) is an economically important, secondary metabolite (30), the end product of the gibberellin (GA) pathway (7, 42). The GAs are a large family of biosynthetically related tetracyclic diterpenes, naturally present in plants in which GA_3 acts as a hormone, regulating many different growth processes (10, 42).

GA_3 is produced industrially by submerged cultivation of the fungus *Gibberella fujikuroi*—the perfect stage of *Fusarium moniliforme* (10). Even though light (23) and extracellular glucose levels (9, 14) can affect the fungal synthesis of GA_3, the latter has been reported to occur only under limited-nitrogen conditions (12, 14). It was recently shown that the onset of GA_3 production can be established before the end of the exponential growth phase when the fungus is cultivated in a complex medium (11). Furthermore, GA_3 synthesis was found in resting cells prepared from cultures harvested at different growth phases (40). However, addition of nitrogen sources to both GA_3-producing cultures and resting cell systems shut off GA_3 formation (11, 12, 14, 40). These studies suggest that ammonium or a product derived from nitrogen metabolism could be the metabolite effector of nitrogen repression of GA_3 synthesis.

Although some enzymatic activities specific to the fungal GA pathway have been described (5, 17), regulatory studies on these activities have not been reported. Little attention has been paid to the physiology of nitrogen metabolism in *G. fujikuroi* and its control (2, 26). The exact mechanism by which nitrogen regulates the fungal GAs synthesis has not been elucidated (10, 14).

Nitrogen catabolite repression has been extensively studied in the filamentous fungi *Neurospora crassa* and *Aspergillus nidulans* (4, 31, 45). Preferentially, these fungi employ ammonium or glutamine as nitrogen sources. The depletion of either of these compounds leads to an increased synthesis of various enzymes which are required for catabolism of several secondary nitrogen sources, such as nitrate, purines, amides, amino acids, and proteins (31, 45). This metabolic adaptation is dependent on the nitrogen regulatory circuit. The latter involves a number of unlinked structural genes, simultaneously controlled by both major and minor regulatory genes as well as by metabolic inducers and nitrogen catabolic repression (4, 31).

Glutamine has been identified as a key metabolic repressor in the nitrogen regulatory circuit (31, 45), although its exact role has not yet been defined. It has been proposed that glutamine interacts directly with the major gene product, converting it into an inactive form and turning off the regulated gene expression (31). Several nitrogen-regulated genes have been cloned, and their transcriptional regulation has been demonstrated (24, 28). Metabolically, glutamine can be considered the final product of nitrogen assimilation (32). This fact confers upon glutamine the ability to regulate nitrogen flow in the cell; therefore, this amino acid may be an intracellular indicator of the state of the extracellular nitrogen content.

In this work, we have studied whether a similar nitrogen control system is also operative in *G. fujikuroi*, governing activities subject to nitrogen regulation, particularly those related to GA_3 synthesis. First, we characterized physiologically GA_3 production in fermentor cultures. The extracellular accumulation of GA_3 and the incorporation of radioactive mevalonic acid into kaurene, by cell extracts, were related to the extracellular nitrogen content. Several enzymatic activities of nitrogen catabolism and assimilation were examined. Second, the participation of glutamine as the metabolic repressor in the nitrogen control system in *G. fujikuroi* was studied. Finally, the effect of ammonium on GA_3 production was determined under conditions in which ammonium conversion into glutamine was inhibited by the presence of L-methionine-DL-sulfoximine (MSX) (38).

MATERIALS AND METHODS

Microorganisms. *G. fujikuroi* ATCC 12616 was maintained at 4°C and periodically subcultured on malt-yeast extract agar slant tubes at 28°C.

Culture conditions. (i) Media. In this work, we used a basal

* Corresponding author.

medium supplemented with 8% glucose and ammonium tartrate as a nitrogen source. The nitrogen concentration was varied according to the experiment. The basal medium had the following composition (in grams per liter of distilled water): KH_2PO_4, 5; $MgSO_4 \cdot 7H_2O$, 0.5; and salts solution, 10 ml. The medium was buffered with 100 mM sodium tartrate, pH 4.5. The salt solution contained (in milligrams per liter) the following: $FeSO_4 \cdot 7H_2O$, 200; $ZnSO_4 \cdot H_2O$, 200; $CaCl_2 \cdot 2H_2O$, 100; $CuSO_4 \cdot 5H_2O$, 20; $CoCl_2 \cdot 6H_2O$, 20; $NaB_4O_7 \cdot 10H_2O$, 20; $Na_2MoO_2 \cdot 2H_2O$, 20; $MnSO_4 \cdot H_2O$, 20; and Na_4-EDTA, 600.

(ii) Shake flask cultures. Cultures were grown in 500-ml conical flasks containing 100 ml of medium with 25 mM nitrogen. Mycelia from 7-day-old slant tubes were suspended with 12 ml of sterile distilled water and suspended with glass beads. The resulting mycelial suspension was employed to inoculate culture flasks (5% [vol/vol]). The flasks were incubated in an orbital shaker at 30°C and agitated at 180 rpm. After 72 h of cultivation, 10 mM ammonium or glutamine was aseptically added, with or without 2 mM MSX (Sigma). Samples (2 ml) were taken every 12 h, the liquid medium was separated by filtration, and GA_3 was quantified.

When the effect of nitrogen sources on mycelial growth was evaluated, the fungus was cultivated in 250-ml conical flasks containing 20 ml of medium with 10 mM nitrogen. The flasks were inoculated, as described above, and incubated at 30°C without agitation. Growth was determined, during the exponential growth phase, at 4 days of incubation.

(iii) Fermentor cultures. These cultures were carried out in a New Brunswick BioFlo IIc fermentor equipped with a 2.5-liter vessel, which was inoculated with a 20-h shake flask culture. Fermentation conditions were as follows: 30°C, agitation at 400 rpm, aeration with sterile air at 1 liter/min (0.4 VVM), and 46 mM nitrogen. As required, a water-soluble silicone antifoaming agent was added.

Preparation of crude cell extracts. Mycelial samples were harvested by filtration through Whatman no. 1 filter paper, washed with distilled water, and frozen at −20°C. The samples were lyophilized and weighed before being ground in a mortar with Pyrex glass powder. The resulting fine powder was suspended for 1 h at 4°C in 8 volumes of extraction buffer (0.1 M phosphate buffer [pH 7.4], 15% glycerol, 5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). The resulting paste was centrifuged at 10,000 × *g* for 30 min at 4°C. The supernatant obtained was centrifuged again as before, and the lipid layer was removed. The supernatant was employed as the source of enzymes and was stored at −20°C until used.

Enzyme assays. NADP-glutamate dehydrogenase (GDH; EC 1.4.1.4) was assayed as described by Fincham (18), and NADH-linked glutamate synthase (GOGAT; EC 1.4.1.14) was assayed as described by Boland and Benny (8). Both activities were determined by following the oxidation of NADPH or NADH at 340 nm. Specific activities were expressed as the absorbance changes per milligram of protein per minute. Glutamine synthetase (GS; EC 6.3.1.2) was assayed by hydroxamate formation (39). Formamidase (EC 3.5.1.9) and urease (EC 3.5.1.5) were assayed by quantity of ammonium released from the respective substrates, as previously described (21, 41). Both the amidases and glutamine synthetase specific activities were expressed as milliunits per milligram of protein. One unit is the amount of enzyme that catalyzes the release of 1 μmol of ammonium or γ-glutamylhydroxamate per min.

The synthesis of kaurene in the cell extracts was assayed in reaction mixtures containing 0.05 μCi of [2-^{14}C]mevalonic acid (5.1 mCi/mmol; Amersham), 2 mM $MgCl_2$, 6 mM ATP, 5 mM dithiothreitol, 10 mM NaF, and 50 mM TES [*N*-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid] buffer (pH 7) in a total volume of 1 ml. The reaction was started by addition of the cell extract and stopped with 0.5 ml of ethanol-acetone (1:1 [vol/vol]) after a 3-h incubation. After three extractions with diethyl ether (3 ml [each]), the dried ether extracts were suspended in 50 μl of benzene-acetone (3:1 [vol/vol]) and chromatographed on silica gel G thin-layer chromatography plates (F-250; Merck) with hexane. To quantify the kaurene produced, the silica in the kaurene region was scraped from the plates and counted by liquid scintillation. The enzymatic activity of the kaurene synthesis was expressed in disintegrations per minute of kaurene produced during the assay per milligram of protein.

All incubations were carried out at 30°C, and each assay was repeated at least twice.

Miscellaneous methods. The ammonium in the culture medium was quantified by the phenol-hypochlorite method as described by Muftic (33); GA_3 was measured fluorimetrically as described by Kavanagh and Kuzel (25). The protein concentration was determined by a modified Lowry method (43). Growth was determined gravimetrically from lyophilized samples or as previously described (16).

Special chemicals. Standards of *ent*-kaurene and the kaurene synthetase inhibitor AMO 1618 [2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenyl piperidine-1-carboxylate] (17) were kindly provided by Peter Hedden, Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Bristol, England.

RESULTS

Physiology of GA_3 production in fermentor cultures. (i) Onset of GA_3 synthesis. Extracellular GA_3 could not be detected until the nitrogen source was totally depleted from the culture medium (Fig. 1A and B). GA_3 appeared 24 h after the depletion of exogenous nitrogen, when the stationary growth phase had been established.

In order to examine the GA_3 pathway, cell extracts (300 μl [equivalent to 1.5 mg of protein]) of *G. fujikuroi* were tested for their ability to incorporate [^{14}C]mevalonic acid into kaurene. [^{14}C]kaurene increased linearly (to 2.5 × 10^3 dpm) with reaction time and protein concentration. This transformation was totally inhibited (0.1 × 10^3 dpm) by the kaurene synthetase inhibitor AMO 1618. The biosynthesis of kaurene almost paralleled fungal cell growth, which remained at a constant level when the stationary phase was reached (Fig. 1B). A low, but significant, synthesis of kaurene was detected in extracts prepared from mycelial samples taken during the exponential growth phase. This basal or constitutive level of activity corresponds to a 0.2% transformation of mevalonic acid into kaurene. Kaurene biosynthesis increased by a factor of 10 after 35 h of cultivation and reached a steady state after 47 h. The maximum was 30 times higher than the basal level, coinciding with high rates of GA_3 production. The increase in the kaurene biosynthetic activities was determined 11 h after the exogenous nitrogen was depleted and 12 h before GA_3 was detected extracellularly in the culture medium.

(ii) Development of formamidase and urease activities. Several enzymatic activities related with the catabolism of secondary nitrogen sources were examined. Although urease showed a basal level during the exponential growth

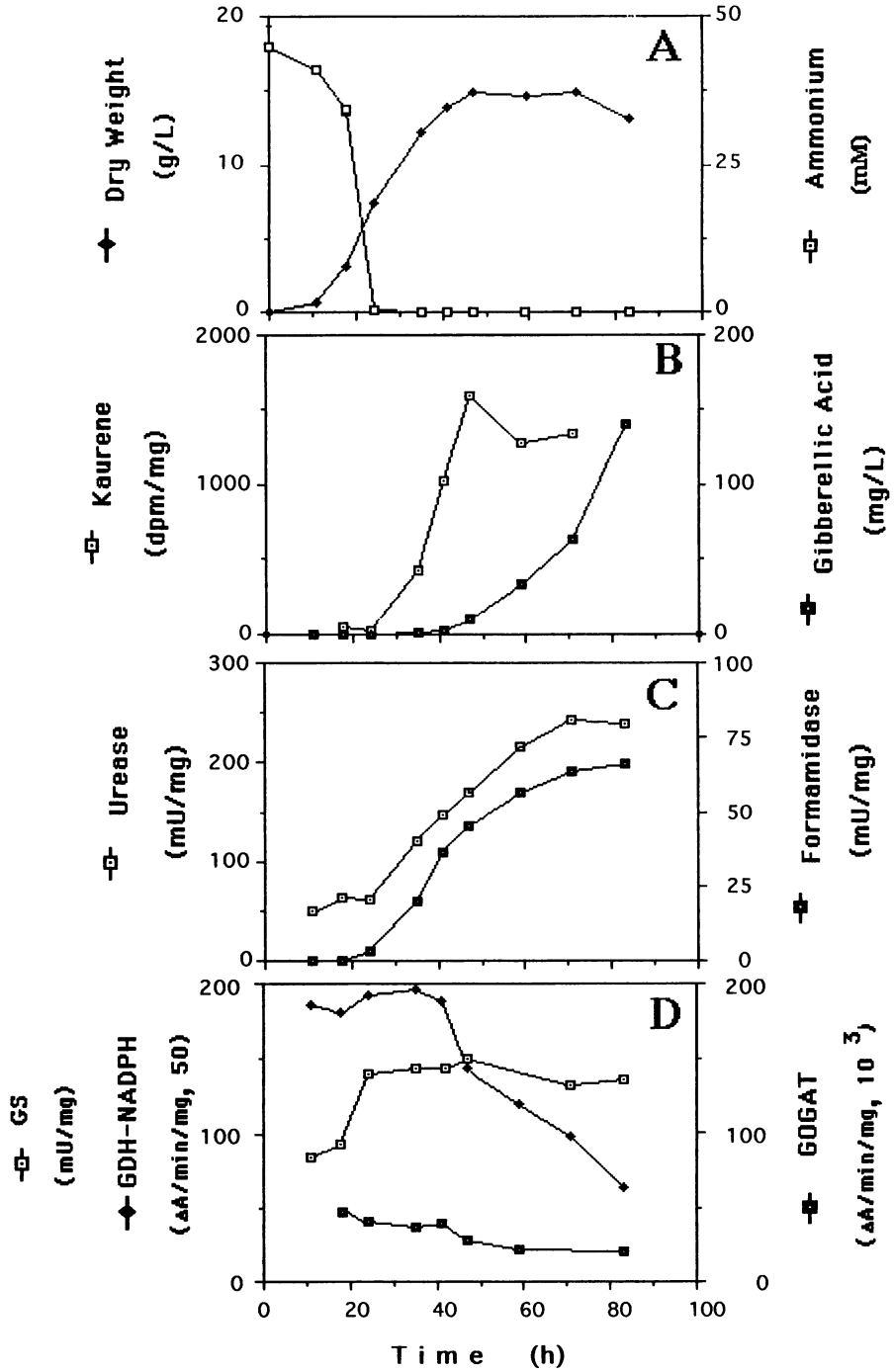

FIG. 1. Physiological characterization of the production of GA_3 by *G. fujikuroi* in nitrogen-limited medium. (A) Fungal growth and ammonium consumption; (B) GA_3 production and [2-^{14}C]mevalonic acid incorporation into kaurene by fungal cell extracts; (C) evolution of formamidase and urease activities; (D) evolution of the main enzymes involved in nitrogen assimilation. ΔA, change in absorbance.

phase, it subsequently increased like formamidase, when the culture neared the end of exponential growth phase (Fig. 1C). Both activities evolved, without substrate induction, in a manner similar to kaurene biosynthesis.

(iii) Development of activities related to nitrogen assimilation. As shown in Fig. 1D, the three enzymatic activities by which the cellular nitrogen is assimilated, i.e., GDH, GS, and GOGAT, were found in *G. fujikuroi*. GOGAT and GDH showed a nearly constant level of activity during the first 40 h of incubation. Both then decreased, coinciding with the onset of the stationary-phase growth. Interestingly, the GS activity doubled immediately after depletion of extracellular ammonium, i.e., before the amidases and GA_3 synthesis could be detected. The maximum GS activity was obtained at that point and remained constant during the rest of the culture.

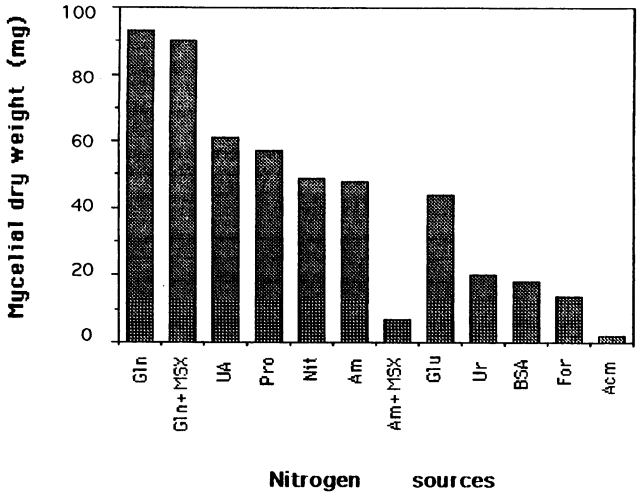

FIG. 2. Mycelial growth of *G. fujikuroi* on different nitrogen sources. The fungus was cultivated during 4 days under the conditions specified in Materials and Methods, before mycelial dry weight was determined. Nitrogen sources (10 mM) were glutamine (Gln), uric acid (UA), proline (Pro), sodium nitrate (Nit), ammonium tartrate (Am), glutamate (Glu), urea (Ur), BSA, formamide (For), and acetamide (Acm). When indicated, 2.5 mM MSX was added.

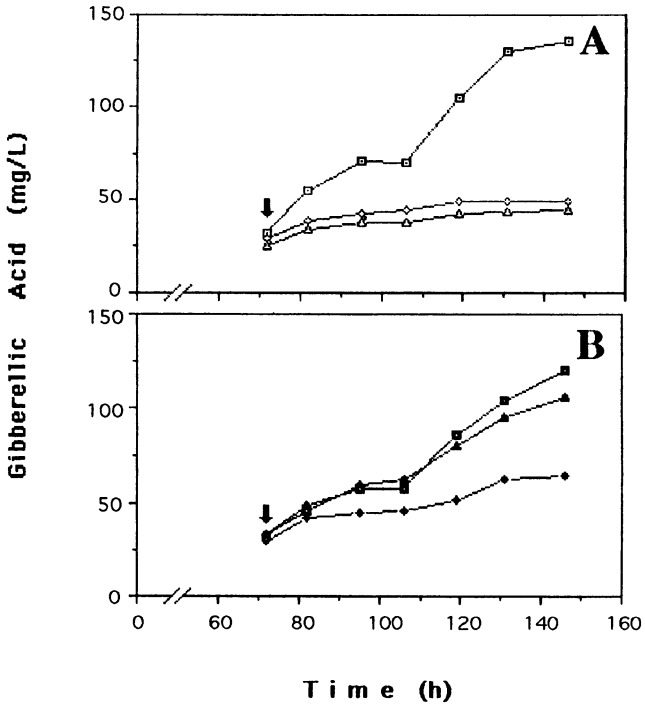

FIG. 3. Effects of 10 mM ammonium and glutamine on extracellular accumulation of GA_3 added without (A; open symbols) or with (B; closed symbols) 2 mM MSX after 72 h of cultivation (arrows) of *G. fujikuroi* in shake flask cultures. Symbols: squares, no addition; triangles, ammonium; diamonds, glutamine.

Participation of glutamine in nitrogen control of the GA_3 production. (i) Effect of nitrogen sources on fungal growth. Among several nitrogen sources tested, glutamine, glutamate, and ammonium supported the highest mycelial growth of *G. fujikuroi*. Nitrogen compounds which need to be previously catabolized, such as proteins (bovine serum albumin [BSA]) and amides (formamide), generally supported lower growth (Fig. 2). Thus, the former compounds can be considered as easily assimilated nitrogen sources in *G. fujikuroi*.

(ii) Effect of nitrogen sources on the activity of nitrogen-regulated activities. Preliminary results showed that formamidase is a nitrogen-regulated enzyme. When mycelium of *G. fujikuroi* (expressing formamidase activity) was transferred to an ammonium-rich culture, formamidase activity decreased. Conversely, activity increased in a medium without nitrogen (not shown). The effect of ammonium, as well as two other easily assimilated nitrogen sources, on several putative nitrogen-regulated activities was then compared (Table 1). The results show that all the activities tested were lower in the presence of glutamine.

To test the possibility of an inhibitory effect of glutamine or ammonium on these activities, enzymatic assays were conducted in the presence of either of these compounds at 10 mM. Under these in vitro conditions, we did not find any inhibitory effect on either formamidase (not shown) or kaurene biosynthetic activities (2.8×10^3 and 2.6×10^3 dpm, respectively).

TABLE 1. Specific activities of enzymes involved in nitrogen metabolism during exponential growth phase on easily assimilated nitrogen sources[a]

Nitrogen source	Sp act			
	GS (mU/mg)	GDH-NADP (ΔA/min/mg)	GOGAT (ΔA/min/mg, 10^3)	Urease (mU/mg)
Ammonium	93 ± 8	3.1 ± 0.4	53 ± 2	63 ± 2
Glutamate	98 ± 6	1.5 ± 0.2	60 ± 3	200 ± 3
Glutamine	28 ± 8	0.9 ± 0.2	41 ± 2	45 ± 5

[a] Fungus was cultured for 20 h in a 500-ml shake flask with 100 ml of medium containing 50 mM nitrogen sources, as indicated, before harvesting. ΔA, change in absorbance.

(iii) Effect of ammonium and glutamine on the production of GA_3. The effect of ammonium and glutamine on the rate of GA_3 production in both the presence and absence of MSX was studied. MSX almost completely inhibited mycelial growth of *G. fujikuroi* in cultures containing ammonium as the sole nitrogen source, while growth occurred normally when glutamine—or a mixture of ammonium and glutamine—was used as a nitrogen source (Fig. 2 and data not shown). Thus, when the inhibitor is present, the fungus employs glutamine as its exclusive nitrogen source. MSX did not inhibit the biosynthesis of kaurene, as assayed in cell extracts of *G. fujikuroi* (2.7×10^3 dpm).

In shake flask cultures, GA_3 was detected after 72 h in the extracellular medium. At this point, addition of several nitrogen sources prevented extracellular GA_3 accumulation (Fig. 3A). The addition of 10 mM ammonium or glutamine stopped extracellular GA_3 accumulation, maintaining GA_3 levels three times less than control cultures, for at least 20 h. In cultures in which only MSX was added, GA_3 production occurred normally, although only 75% of the hormone production was achieved compared with that in the control without MSX (Fig. 3). As expected, the repressive effect of ammonium was prevented by MSX (Fig. 3), while glutamine repression occurred both with and without MSX. Finally, when ammonium, glutamine, and MSX were combined, GA_3 production was inhibited (results not shown).

DISCUSSION

Nitrogen control of GA_3 production is a good system to study the regulation of secondary metabolites. This compound originates exclusively from carbon precursors (7, 10, 42) and therefore is not directly related to nitrogen metabolism, as are many other secondary metabolites, e.g., antibiotics (1, 37).

This work investigated the mechanism of nitrogen control on the GA_3 pathway in *G. fujikuroi*. A low but significant level of kaurene biosynthetic activity was present during the exponential growth phase. Kaurene synthesis is dependent on the activity of the isoprenoid pathway, as well as that of the first GA_3 pathway enzyme, kaurene synthetase (7, 10, 17, 42). Our results confirm previous studies which indicated that the isoprenoid and GA pathways were constitutively expressed (6, 35, 36, 40). These results imply that GA_3 is synthesized at very low levels during the exponential growth phase and could be responsible for several fungal processes, as already suggested (34, 35).

Several enzymatic activities related to nitrogen metabolism were expressed simultaneously with the onset of GA_3 synthesis. This strongly suggests that all of these activities are nitrogen regulated and may be subject to the same type of control. The development of formamidase and urease activities was similar to that reported in other fungi (21, 22, 41).

Nitrogen assimilation in *G. fujikuroi* exhibited similarities to those described for *N. crassa* (31, 32). Under conditions of ammonium sufficiency, GDH-NADPH and an octameric form of GS are involved in ammonium assimilation in *N. crassa* (27, 29), while under nitrogen limitation, ammonium is assimilated by GS and GOGAT (20, 24). Considering that the levels of these activities were lower when glutamine was the nitrogen source, we propose that this amino acid may well be the final product of nitrogen assimilation in *G. fujikuroi*.

Glutamine has been reported to cycle in *N. crassa* (32). This cycling is required to drive an effective carbon flow to support growth and would facilitate the allocation of nitrogen and/or carbon according to cellular demands. If a similar cycle operates in *G. fujikuroi*, the effect of MSX on GA_3 production could be explained. For example, when GS is inhibited by MSX, the cycle stops and energy charge increases. This would result in a decrease in the carbon flow and consequently on the availability of precursors for biosynthesis (19). Clearly, more detailed studies are necessary to elucidate this hypothesis.

Glutamine does not act directly on any specific activity of the GA pathway, at least not on enzymes responsible for the synthesis of kaurene. Two possibilities may explain the role of this amino acid on nitrogen control. First, glutamine may affect enzyme stability, as proposed in the regulation of protein turnover rates for skeletal muscle (44). Second, glutamine could function at the transcriptional level, as proposed for *N. crassa* and *A. nidulans* (31, 45). At this level, several nitrogen-regulated activities are controlled in these fungi (4, 24, 28). If *G. fujikuroi* has the same type of nitrogen control system, mutants affected in the major regulatory gene could be selected (3, 15, 31, 45). The activities involved in both nitrogen catabolism and GA_3 synthesis would no longer be nitrogen regulated in these mutants. Considering that *G. fujikuroi* and *N. crassa* are closely related ascomycetes (13), it is reasonable that a similar mechanism of nitrogen control could operate in both fungi. The fact that several putative nitrogen-regulated activities showed low levels when glutamine was employed as the sole nitrogen source suggests that glutamine is the repressor of activities subject to nitrogen control in *G. fujikuroi*.

ACKNOWLEDGMENTS

We are indebted to Etienne Odier and German Aroca for helpful comments and contribution concerning the design of the initial experiments. We also thank Lenka Torres for skillful technical assistance, Mylene Maureira and Ricardo San Martin for advice on fermentor runs, and Gloria Portilla for her help in the kaurene assay setup. The continuous interest and help during this work of Manuel Pinto and Julio Retamales are also greatly acknowledged. We are grateful to Alex Crawford for careful English review of the manuscript.

This research was supported by Fundación Andes grant C-11001.

REFERENCES

1. **Aharonowitz, Y.** 1980. Nitrogen metabolite regulation of antibiotic biosynthesis. Annu. Rev. Microbiol. **34:**209–233.
2. **Allam, A. M., and T. A. Elzainy.** 1971. Purine catabolism in *Fusarium moniliforme*. J. Gen. Microbiol. **63:**183–187.
3. **Arst, H. N., and D. J. Cove.** 1973. Nitrogen metabolite repression in *Aspergillus nidulans*. Mol. Gen. Genet. **126:**111–142.
4. **Arst, H. N., and C. Scazzocchio.** 1985. Formal genetics and molecular biology of the control gene expression in *Aspergillus nidulans*, p. 309–343. *In* J. W. Bennett and L. L. Lasure (ed.), Gene manipulations in fungi. Academic Press, London.
5. **Asham, P. J., A. Mackenzie, and P. M. Brambley.** 1990. Characterization of *ent*-kaurene oxidase activity from *Gibberella fujikuroi*. Biochim. Biophys. Acta **1036:**151–157.
6. **Avalos, J., A. Mackenzie, D. S. Nelki, and P. M. Bramley.** 1988. Terpenoid biosynthesis in cell extracts of wild type and mutant strains of *Gibberella fujikuroi*. Biochim. Biophys. Acta **966:**257–265.
7. **Bearder, J. R.** 1983. *In vivo* diterpenoid biosynthesis in *Gibberella fujikuroi*. The pathway after *ent*-kaurene, p. 251–387. *In* A. Crozier (ed.), The biochemistry and physiology of gibberellins, vol. 1. Praeger, New York.
8. **Boland, M. J., and A. G. Benny.** 1977. Enzymes of nitrogen metabolism in legumes nodules. Purification and properties of NADH-dependent glutamate synthase from lupin nodules. Eur. J. Biochem. **79:**355–362.
9. **Borrow, A., S. Brown, E. G. Jefferys, R. H. Kessell, E. C. Loyd, P. B. Lloyd, A. Rothwell, B. Rothwell, and J. C. Swait.** 1964. The kinetics of metabolism of *Gibberella fujikuroi* in stirred culture. Can. J. Microbiol. **10:**407–444.
10. **Brückner, B., and D. Blechschmidt.** 1991. The gibberellin fermentation. Crit. Rev. Biotecnol. **11:**1163–1192.
11. **Brückner, B., and D. Blechschmidt.** 1991. Nitrogen regulation of gibberellins biosynthesis in *Gibberella fujikuroi*. Appl. Microbiol. Biotechnol. **53:**646–650.
12. **Bu'lock, J., R. Detroy, Z. Hostalek, and A. Munin-al-Shakarchi.** 1974. Regulation of secondary biosynthesis in *Gibberella fujikuroi*. Trans. Br. Mycol. Soc. **62:**377–389.
13. **Burnett, J. H.** 1975. Principal fungi studied genetically, p. 321–328. *In* J. H. Burnett (ed.), Mycogenetics. John Wiley & Sons, Inc., London.
14. **Candau, R., J. Avalos, and E. Cerdá-Olmedo.** 1992. Regulation of gibberellin biosynthesis in *Gibberella fujikuroi*. Plant Physiol. **100:**1184–1188.
15. **Cohen, B. L.** 1972. Ammonium repression of extracellular protease in *Aspergillus nidulans*. J. Gen. Microbiol. **71:**293–299.
16. **Cotoras, M., and E. Agosin.** 1992. Regulatory aspects of endoglucanase production by the brown-rot fungus *Gloeophyllum trabeum*. Exp. Mycol. **16:**253–260.
17. **Fall, R. R., and C. West.** 1971. Purification and properties of kaurene synthetase from *Fusarium moniliforme*. J. Biol. Chem. **246:**6913–6929.
18. **Fincham, J. R. S.** 1950. Mutant strains of *Neurospora* deficient in aminating ability. J. Biol. Chem. **181:**61–73.
19. **Hernández, G., and J. Mora.** 1986. Glutamine synthesis regu-

lates sucrose catabolism in *Neurospora crassa*. J. Gen. Microbiol. **132**:3315–3323.
20. **Hummelt, G., and J. Mora.** 1980. NADH-dependent glutamate synthase and nitrogen metabolism in *Neurospora crassa*. Biochem. Biophys. Res. Commun. **92**:127–133.
21. **Hynes, M. J.** 1970. Induction and repression of amidase enzymes in *Aspergillus nidulans*. J. Bacteriol. **103**:482–487.
22. **Hynes, M. J.** 1974. Effects of ammonium, L-glutamate, and L-glutamine on nitrogen catabolism in *Aspergillus nidulans*. J. Bacteriol. **120**:1116–1123.
23. **Johnson, S. W., and R. C. Coolbaugh.** 1990. Light-stimulated gibberellin biosynthesis in *Gibberella fujikuroi*. Plant Physiol. **94**:1696–1701.
24. **Johnstone, I. L., P. C. McCabe, P. Greaves, S. J. Gurr, G. E. Cole, M. A. D. Brow, S. E. Unkles, A. J. Clutterbuck, J. R. Kinghorn, and M. A. Innis.** 1990. Isolation and characterization of the *crnA-niiA-niaD* gene cluster for nitrate assimilation in *Aspergillus nidulans*. Gene **90**:181–192.
25. **Kavanagh, F., and N. Kuzel.** 1958. Gibberellins assays. Fluorimetric determination of gibberellic and gibberellenic acids in fermentation products, commercial formulations and purified materials. Agric. Food Chem. **6**:459–463.
26. **Klittich, C. J. R., and J. F. Leslie.** 1989. Chlorate-resistant, nitrate-utilizing (*crn*) mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). J. Gen. Microbiol. **135**:721–727.
27. **Lara, M., L. Blanco, M. Campomanes, E. Calva, R. Palacios, and J. Mora.** 1982. Physiology of ammonium assimilation in *Neurospora crassa*. J. Bacteriol. **150**:105–112.
28. **Lee, H., Y. H. Fu, and G. A. Marzluf.** 1990. Molecular cloning and characterization of *alc*, the gene encoding allantoicase of *Neurospora crassa*. Mol. Gen. Genet. **222**:140–144.
29. **Lomnitz, A., J. Calderón, G. Hernández, and J. Mora.** 1987. Functional analysis of ammonium assimilation enzymes in *Neurospora crassa*. J. Gen. Microbiol. **133**:2333–2340.
30. **Martin, G. C.** 1983. Commercial uses of gibberellins, p. 395–444. *In* A. Crozier (ed.), The biochemistry and physiology of gibberellins, vol. 1. Praeger, New York.
31. **Marzluf, G. A.** 1981. Regulation of nitrogen metabolism and gene expression in fungi. Microbiol. Rev. **54**:293–304.
32. **Mora, J.** 1991. Glutamine metabolism and cycling in *Neurospora crassa*. Microbiol. Rev. **45**:437–461.
33. **Muftic, M. K.** 1964. A new phenol-hypochlorite reaction for ammonia. Nature (London) **201**:622–623.
34. **Nakamura, T., Y. Kawanabe, E. Takayima, N. Takahashi, and T. Murayama.** 1978. Effects of auxin and gibberellin on conidial germination of *Neurospora crassa*. Plant Cell Physiol. **19**:705–709.
35. **Nakamura, T., K. Mitsuoka, M. Sugano, K. Tomita, and T. Murayama.** 1985. Effects of auxin and gibberellin on conidial germination and elongation of young hyphae in *Gibberella fujikuroi* and *Penicillium notatum*. Plant Cell Physiol. **26**:1433–1437.
36. **Nes, W. D., P. K. Hanners, and E. J. Parish.** 1986. Control of fungal sterol C-24 transalkylation: importance to developmental regulation. Biochem. Biophys. Res. Commun. **139**:410–415.
37. **Nüesch, J., J. Heim, and H. J. Treichler.** 1987. The biosynthesis of sulfur-containing β-lactam antibiotics. Annu. Rev. Microbiol. **41**:51–75.
38. **Premakumar, R., G. J. Sorger, and D. Gooden.** 1980. Repression of nitrate reductase in *Neurospora* studied by using L-methionine-DL-sulfoximine and glutamine auxotroph *gln-1b*. J. Bacteriol. **143**:411–414.
39. **Rowe, W. B., R. A. Ronzio, V. P. Wellner, and A. Meister.** 1970. Glutamine synthetase (sheep brain). Methods Enzymol. **17A**: 900–910.
40. **Rybakov, Y. A., and G. I. Bourd.** 1991. Nitrogen regulation of gibberellin biosynthesis enzyme complex in *Fusarium moniliforme*. J. Biotechnol. **21**:219–228.
41. **Scazzocchio, C., and A. J. Darlington.** 1968. The induction and repression of the enzymes of purine breakdown in *Aspergillus nidulans*. Biochim. Biophys. Acta **166**:557–568.
42. **Takahashi, N., I. Yamaguchi, and H. Yamane.** 1986. Gibberellins, p. 57–151. *In* N. Takahashi (ed.), Chemistry of plant hormones. CRC Press, Boca Raton, Fla.
43. **Tan, M. U. L., M. K.-H. Chan, and J. N. Saddler.** 1984. A modification of the Lowry method for detecting proteins in media containing lignocellulosic substrates. Biotechnol. Lett. **6**:199–204.
44. **Watford, M.** 1989. Does glutamine regulate skeletal muscle protein turnover? Trends Biochem. Sci. **14**:1–2.
45. **Wiame, J. M., M. Grenson, and H. N. Arst.** 1985. Nitrogen catabolite repression in yeast and filamentous fungi. Adv. Microbiol. Physiol. **29**:1–88.