JOURNAL OF BACTERIOLOGY, Aug. 1984, p. 583–589
0021-9193/84/080583-07$02.00/0

Vol. 159, No. 2

Participation of an Extracellular Deaminase in Amino Acid Utilization by *Neurospora crassa*

RUTH MASON DEBUSK* AND SUSAN OGILVIE

Department of Biological Science, Florida State University, Tallahassee, Florida 32306

Received 20 December 1983/Accepted 14 May 1984

A strain of *Neurospora crassa* defective in amino acid transport can utilize a variety of amino acids for growth when readily metabolizable nitrogen is limiting. Growth is accompanied by the production of an extracellular deaminase that converts the amino acid to its respective keto acid plus equimolar quantities of utilizable nitrogen in the ammonium ion form. Production of the deaminase is subject to ammonium repression. The relationship between the ability of an amino acid to trigger deaminase production and the presence of particular amino acid permease deficiencies is complex. Four classes of amino acids have been defined with respect to this relationship. The existence of multiple extracellular deaminases is discussed.

Ammonium, glutamate, and glutamine are preferred nitrogen sources for most procaryotes and lower eucaryotes. In the absence of such readily metabolizable nitrogenous compounds, however, a variety of secondary compounds can serve as sole sources of nitrogen for these organisms. Many of the enzymes and permeases involved in the utilization of secondary nitrogen sources are subject to nitrogen metabolite repression, which insures that these activities are functional only when supplies of the preferred nitrogen sources are limited. Excellent reviews of secondary nitrogen utilization and its regulation are available for both procaryotes and the lower eucaryotes (12, 17, 19).

Utilization of amino acids as secondary nitrogen sources involves transport of the amino acid into cells followed by enzymatic removal of the alpha-amino nitrogen through deamination or transamination. In the filamentous fungus *Neurospora crassa*, transport is accomplished by three constitutive amino acid permeases and three specialized permeases. The constitutive permeases include a neutral amino acid-specific (N) system, a basic amino acid-specific (B) system, and a general (G) system that can transport all classes of amino acid (5, 7, 18, 20, 22, 23, 37). Three additional systems that function during advanced stages of development have been proposed for the transport of the imino acid proline, of acidic amino acids, and of the sulfur-containing amino acid methionine during sulfur limitation (10, 24, 25, 38, 40). Subsequent enzymatic degradation can occur through a generalized transamination mechanism whereby the alpha-amino group of the amino acid is transferred to a receptor keto acid. Alternatively, degradation may involve deamination which can be highly specific for particular amino acids, as in the case of the amino acid lyases, or which may act upon a broad range of amino acids, as is the case of the amino acid oxidases (2, 21, 31, 32, 34).

We have previously reported an interesting example of amino acid utilization involving the *pmn*; *pmb*; *pmg* strain of *N. crassa* that is unable to transport the basic amino acid arginine into cells due to mutations in each of its constitutive amino acid permeases (8). Arginine can, however, serve as the sole source of nitrogen for this strain. Growth is correlated with the production of an extracellular deaminase. The enzyme is produced only in the absence of a primary nitrogen source and only by those strains defective in general amino acid permease activity.

In this paper, we extend the original observations to demonstrate that extracellular deaminase activity is produced by this transport-defective strain in response to amino acids in general rather than to arginine in particular, that enzyme production can be elicited only in the absence of a readily metabolizable nitrogen source, and that the various amino acids differ in their requirement for defective general permease activity as a signal in triggering production of the deaminase.

MATERIALS AND METHODS

Strains. The *pmn*; *pmb*; *pmg* strain of *N. crassa* is defective in constitutive amino acid transport by virtue of three single-gene mutations. The *pmn* mutation eliminates activity of the N system, the *pmb* mutation eliminates B system permease activity, and the *pmg* mutation eliminates the activity of the G system permease (15, 28, 29). The triple mutant was constructed in this laboratory and deposited with the Fungal Genetics Stock Center (Humboldt State University, Arcata, Calif.) as strain FGSC 2606. This strain was backcrossed four times to the wild-type strain 74, the resultant eight progeny were recovered through tetrad analysis, and amino acid transport analysis was used to assign genotypes to each.

Growth of cultures. Strains were maintained on solidified 1× Vogel medium N (36) containing NH_4NO_3 as the nitrogen source, 2% sucrose as the carbon source, and 1.5% agar. The culture storage and growth regimens are described by DeBusk and DeBusk (7).

Isolation of the *pmn*; *pmb*; *pmg*; *lox* strain. Conidia from a single 125-ml Erlenmeyer flask of the *pmn*; *pmb*; *pmg* strain grown in 25 ml of the solid medium described above were harvested aseptically into 25 ml of sterile distilled water. Mycelial fragments were removed by passing the cell suspension through a filter apparatus containing cheesecloth and glass wool. A 7-ml sample of the cell suspension (10^7 conidia per ml) was irradiated with UV light to 10% survival, placed in the dark at room temperature for 45 min to prevent photoreactivation, and added to 875 ml of sterile, tempered 1× Vogel medium N containing equimolar KNO_3 in place of the NH_4NO_3, a 0.4 mM concentration of the amino acid analog *para*-fluorophenylalanine (FPA), 1.5% L-sorbose,

* Corresponding author.

0.15% sucrose, and 1.5% agar. The mixture was then poured into petri plates. Plates were incubated at 35°C; colonies were isolated over a 2-day period into 1× Vogel medium N containing KNO_3 as the nitrogen source, 0.4 mM FPA, and 2% sucrose. Those isolates that were resistant to the growth-inhibitory effects of FPA were backcrossed to the parental strain, and FPA-resistant isolates were obtained, again backcrossed to the *pmn*; *pmb*; *pmg* strain, and reisolated. An isolate found to be FPA resistant with an accompanying deficiency in L-amino acid oxidase activity was designated *pmn*; *pmb*; *pmg*; *lox*.

Growth assays. Liquid growth assays were conducted in duplicate at 35°C in 125-ml Erlenmeyer flasks containing 25 ml of medium. After 72 h, the mycelial pads were removed, dried, and weighed. When amino acid utilization was tested, the ammonium nitrate of Vogel medium N was removed to produce a nitrogen-free basal medium to which an amino acid was added to a final concentration of 15 mM. When FPA resistance was tested, the basal medium used was Vogel medium N containing 25 mM NH_4NO_3 or KNO_3.

Deaminase and competition assays. The conversion of amino acid to keto acid was measured by using a microassay in which dialyzed culture medium was incubated with radiolabeled phenylalanine, and the keto acid product was detected. The assay is a modification of that of Woodward and Cirillo (7, 39). To determine whether an amino acid could potentially serve as a substrate for deaminase action, the test amino acid was added at 100 times the concentration of the radiolabeled phenylalanine, and the ability to interfere with phenylpyruvate production was measured. Culture medium from the *pmn*; *pmb*; *pmg* strain grown in the presence of L-arginine as the sole nitrogen source was used as the source of deaminase for the competition assays.

Chromatography. For the comparison of the reaction products generated by deaminase action on arginine with those of L-amino acid oxidase and arginase, the deaminase was produced in response to arginine. The enzymes were incubated in radiolabeled arginine, and the reaction products were separated on Silica Gel 60 F plates with 1-butanol–pyridine–acetic acid–water (75:50:15:55) as the solvent and visualized by autoradiography. Radiolabeled arginine, ornithine, and urea were the standards. Additionally, reaction products from deaminase conversion were mixed with those from arginase or L-amino acid oxidase conversion and chromatographed as single samples.

Similarly, the reaction product generated by the arginine-induced deaminase from radiolabeled leucine or phenylalanine was compared with that produced by L-amino acid oxidase. Polyethyleneimine-cellulose F sheets were used, and the solvent was 1-butanol–acetic acid–water (80:20:20). For each substrate, each set of reaction products was spotted separately and in combination. The radiolabeled leucine keto acid alpha-ketoisocaproate and the radiolabeled phenylalanine keto acid phenylpyruvate were used as controls in addition to radiolabeled leucine and phenylalanine.

To compare the reaction products generated from radiolabeled arginine, leucine, or phenylalanine by deaminase activity produced in response to different amino acids, we prepared culture filtrates of the *pmn*; *pmb*; *pmg* strain grown in the following amino acids as sole nitrogen sources: L-arginine, L-histidine, L-glycine, L-citrulline, L-serine, L-valine, L-methionine, L-proline, L-phenylalanine, and L-leucine. Chromatographic analysis was carried out as described above.

Chemicals. All chemicals and enzymes were of the highest quality available from Sigma Chemical Co. (St. Louis, Mo.). L-Amino acid oxidase was isolated from *Crotalus adamanteus*; arginase was isolated from bovine liver. Radiolabeled amino acids were purchased from Schwartz/Mann (Cambridge, Mass.). Radiolabeled alpha-ketoisocaproate was obtained from Amersham Corp. (Arlington Heights, Ill.).

RESULTS

Utilization of amino acids as sole sources of nitrogen. Wild-type strain 74a and the *pmn*; *pmb*; *pmg* strain, which contains mutations in each of the constitutive amino acid transport systems, were investigated for their ability to utilize a variety of amino acids as sole sources of nitrogen. Amino acids share a common configuration at the alpha-carbon but differ in the composition of the side chain, or R group, attached to the alpha-carbon. Amino acids are classified as basic, acidic, or neutral based on the charge contributed by the R group. Several representatives of each of these three major classes of amino acid were tested: lysine and arginine (basic), glutamic acid (acidic), phenylalanine and tryptophan (aromatic nonpolar neutral), leucine and methionine (long-chain nonpolar neutral), alanine and valine (short-chain nonpolar neutral), glycine and serine (short-chain polar neutral), and citrulline, asparagine, and glutamine (long-chain polar neutral). Histidine exists as a mixture of basic and neutral forms at the pH of the growth medium (5.8). Proline is an imino acid and lacks a free alpha-amino group.

The *pmn*; *pmb*; *pmg* strain grew on all amino acids that supported growth of the wild type. The amino acids varied, however, in the extent to which each could serve as the sole nitrogen source for either strain (Fig. 1a). The D-stereoisomers of phenylalanine and arginine, along with the L-stereoisomers of lysine and tryptophan, did not support growth. L-Histidine and glycine were utilized very poorly. The neutral amino acids serine, valine, citrulline, phenylalanine, methionine, and leucine allowed limited growth. Alanine, a neutral amino acid, supported growth well. The neutral amino acids glutamine and asparagine, the basic amino acids ornithine and arginine, and the acidic amino acid glutamic acid were all excellent nitrogen sources for both strains.

Detection of an extracellular deaminating activity. The *pmn*; *pmb*; *pmg* strain, but not the wild-type strain, previously was shown to produce an extracellular deaminase in response to L-arginine as the sole nitrogen source (8). Since this transport-deficient strain was capable of utilizing a number of other amino acids as sole nitrogen sources, we assayed each culture filtrate to determine whether these amino acids likewise resulted in production of extracellular deaminating activity. Our assay detects the conversion of amino acid to its respective keto acid; the test substrate used was L-phenylalanine. Similar results were obtained with each medium when L-leucine or L-arginine was the substrate and are, therefore, assumed to reflect the ability of a component of the culture filtrate to convert an amino acid to a keto acid and not to be restricted to phenylalanine conversion.

The transport-defective strain produced activity in response to all L-amino acids except proline, tryptophan, glutamic acid, glutamine, and asparagine (Fig. 1b). No activity was elicited in response to proline or to D-amino acids. In contrast, the wild-type strain did not produce activity except in response to particular neutral amino acids, each of which supported growth poorly (Fig. 1a).

Effect of ammonium on deaminase production. Production of extracellular deaminase by the *pmn*; *pmb*; *pmg* strain in response to L-arginine is eliminated when ammonium chloride is simultaneously present in the growth medium (8).

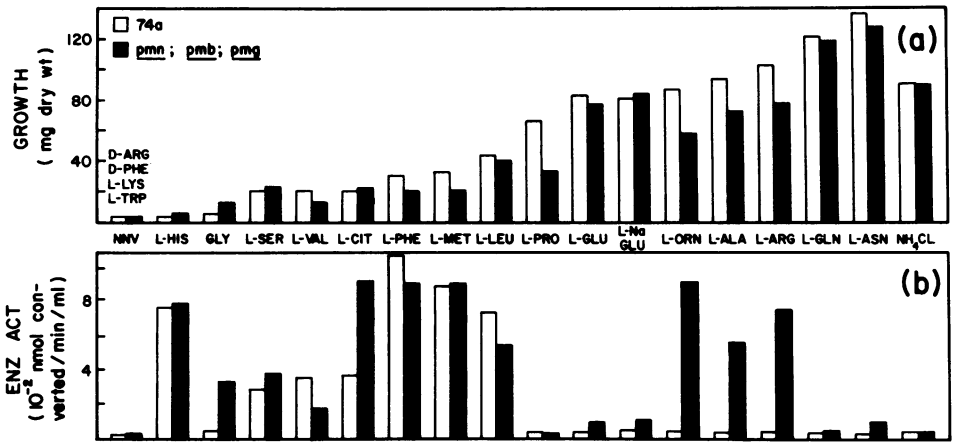

FIG. 1. Growth (a) and production of extracellular deaminase activity (Enz Act) (b) by the wild-type and *pmn*; *pmb*; *pmg* strains in the presence of various amino acids as sole nitrogen sources. All media consisted of nitrogen-free Vogel's medium plus a 15 mM nitrogen source as indicated. Growth assays were performed at 35°C for 72 h. Deaminase assays were performed at 37°C for 2 h.

Since ammonium is known to cause nitrogen metabolite repression, we were interested in whether the deaminase activities produced in response to different amino acids were each subject to ammonium repression. No activity was produced by either strain in response to any amino acid in the presence of ammonium (data not shown).

Deaminase production in response to loss of particular permease activities. We examined whether ability of the *pmn*; *pmb*; *pmg* strain to produce deaminase activity could be correlated with loss of function of particular amino acid transport systems. Eight strains, representing all possible combinations of the amino acid transport mutations, were tested for production of activity in response to arginine, ornithine, and alanine, which elicited activity from the transport-defective strain, but not the wild-type strain, to asparagine and glutamine, which failed to elicit production by either strain, and to histidine, which caused production by both strains (Fig. 2). All strains produced activity in response to histidine, none produced activity in the presence of asparagine and glutamine (data not shown), and, whereas any strain defective for G system activity produced activity in response to arginine, only the triply defective strain could do so in response to ornithine or alanine.

Effect of the *lox* mutation on deaminase production. Extracellular deaminase activity can be produced in response to the amino acid analog FPA with an accompanying inhibition of growth due to deaminase conversion of FPA to its keto acid, which is then transported and regenerated as FPA (6). Ammonium was the nitrogen source for these earlier studies. A *pmn*; *pmb*; *pmg*; *lox* mutant strain resistant to FPA and defective for deaminase activity under the standard FPA-ammonium conditions was isolated. Since amino acids induced a deaminating activity when ammonium was removed from the growth medium, the *pmn*; *pmb*; *pmg*; *lox* strain was additionally tested for its ability to produce deaminase in the absence of ammonium. Figure 3 shows that this strain was unable to produce activity in response to FPA when ammonium was present but could produce activity when ammonium was absent.

These data suggested that the *lox* mutation may be involved with production of deaminase activity under nitrogen-sufficient conditions in response to FPA and perhaps other analogs but may not interfere with deaminase production in response to amino acids when nitrogen is limiting. We then examined the effect of this mutation on enzyme produc-

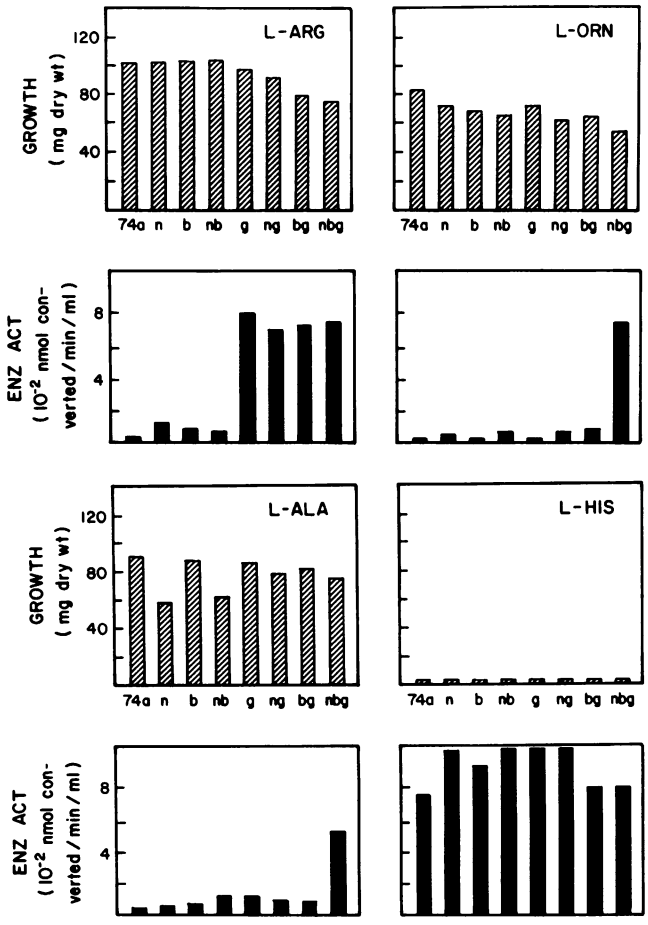

FIG. 2. Growth and production of extracellular deaminase activity (Enz Act) by the wild-type and amino acid transport-defective strains in response to amino acids as sole nitrogen sources. The strains are defective for amino acid transport activity as follows: wild-type (strain 74a), no defects; *pmn* (n), N system defective; *pmb* (b), B system defective; *pmn*; *pmb* (nb), N system defective and B system defective: *pmg* (g), G system defective; *pmn*; *pmg* (ng), N system defective and G system defective; *pmn*; *pmb*; *pmg* (nbg), N system defective, B system defective, and G system defective. Growth and deaminase assays were conducted as for Fig. 1.

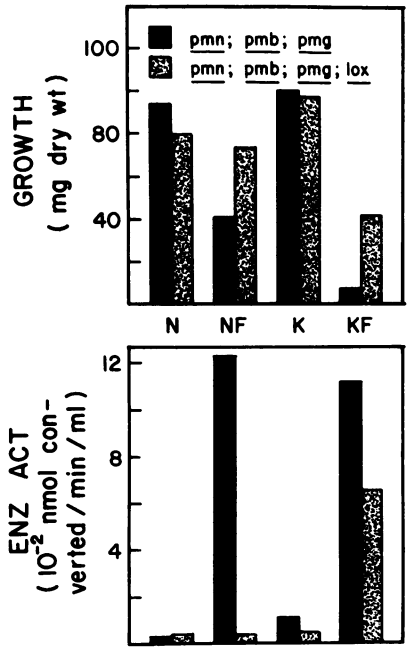

FIG. 3. Growth and production of extracellular deaminase activity (Enz Act) by the *pmn*; *pmb*; *pmg* and *pmn*; *pmb*; *pmg*; *lox* strains in response to FPA in the presence or absence of ammonium. The various media were Vogel medium containing 25 mM NH_4Cl (N), Vogel medium containing 25 mM NH_4Cl plus 0.4 mM FPA (NF), Vogel medium containing 25 mM KNO_3 (K), or Vogel medium containing 25 mM KNO_3 plus 0.4 mM FPA (KF). Growth and deaminase assays were conducted as for Fig. 1.

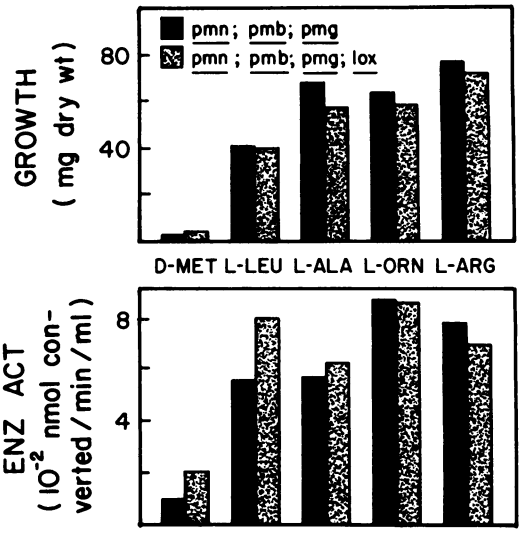

FIG. 4. Growth and production of extracellular deaminase activity (Enz Act) by the *pmn*; *pmb*; *pmg* and *pmn*; *pmb*; *pmg*; *lox* strains in response to amino acids as sole nitrogen sources. All media consisted of nitrogen-free Vogel medium plus 15 mM amino acid. Growth and deaminase assays were conducted as for Fig. 1.

tion in response to representatives of the different classes of amino acids when presented as sole sources of nitrogen. The mutation had no effect on deaminase production under these conditions (Fig. 4).

Evidence that the deaminase is an L-amino acid oxidase. The preliminary findings with the arginine-induced enzyme demonstrated that its activity was not specific for arginine and led us to suspect that arginine, as an amino acid, may be eliciting production of an L-amino acid oxidase that could release the alpha-amino group from the amino acid molecule, thereby providing the nitrogen required for growth. We tested the oxidase hypothesis by determining that the activity produced in response to arginine was not due to the arginine-degradative enzyme arginase and, further, that the activity produced in response to each amino acid generated reaction products identical to those produced by a known L-amino acid oxidase.

Arginase degrades arginine to the nitrogen sources ornithine and urea and, like the amino acid conversion activity we reported previously, is produced in response to arginine under nitrogen-limiting conditions (10, 35). Since the urea generated by arginase can readily be utilized as a nitrogen source by the *pmn*; *pmb*; *pmg* strain (data not shown), arginase could contribute significantly to the ability of this strain to use arginine. We examined the culture filtrate for its ability to produce urea and compared the reaction products with those generated by arginase isolated from bovine liver. No urea was produced when radiolabeled arginine was incubated with culture filtrate from the *pmn*; *pmb*; *pmg* strain grown in the presence of arginine. Furthermore, the product that was generated was chromatographically identical with that generated by a standard L-amino acid oxidase isolated from snake venom (data not shown).

We also tested the ability of the arginine-induced enzyme to generate keto acid from the neutral amino acid L-leucine. The reaction product was chromatographically identical with that produced by the known L-amino acid oxidase and with the alpha-ketoisocaproate control. Similarly, the L-phenylalanine reaction product generated by the arginine-induced enzyme co-chromatographed with that of the control oxidase (data not shown).

To determine whether the arginine-induced enzyme had a broad range of amino acid substrates, the radiolabeled neutral aromatic amino acid L-phenylalanine and the neutral aliphatic amino acid L-leucine were used as substrates, and several unradiolabeled D- and L-amino acids were tested for their ability to interfere with the conversion of the radiolabeled amino acid to its keto acid. Whereas all of the L-stereoisomers were effective in preventing conversion, neither the D-amino acids nor L-proline was effective (Table 1).

We were further interested in whether the activities produced in response to the various classes of amino acids displayed in Fig. 1 could convert amino acids in general to their respective keto acids or were limited to converting that amino acid, or class of amino acid, that elicited its production. Therefore, we prepared culture filtrates of the *pmn*; *pmb*; *pmg* strain grown in the presence of different amino acids as sole nitrogen sources and examined the ability of these filtrates to convert arginine, phenylalanine, and leucine to their respective keto acids. In each case, the extent of conversion of these amino acids to their keto acids was within 1% of the values obtained with both the control L-amino acid oxidase and with the arginine-induced enzyme (data not shown).

DISCUSSION

Deaminase activity that converts amino acids to keto acids is produced by the *pmn*; *pmb*; *pmg* strain in response to a wide range of L-amino acids. Neither D-amino acids nor the imino acid proline allows production or serves as a sub-

TABLE 1. Inhibition by various amino acids of the amino acid-to-keto acid conversion activity of the arginine-induced enzyme[a]

Substrate	Inhibitor	% Inhibition[b]
L-Phe	L-Phe	94
	D-Phe	0
	D-Lys	0
	D-Met	0
	L-Trp	86
	L-Lys	95
	L-His	92
	L-Leu	92
	L-Pro	33
	L-Glu	12
	L-Arg	91
	L-Orn	87
L-Leu	L-Phe	87
	D-Phe	0
	D-Lys	0
	D-Met	0
	L-Trp	86
	L-Lys	87
	L-His	90
	L-Leu	88
	L-Pro	18
	L-Glu	20
	L-Arg	82
	L-Orn	84

[a] The inhibitor concentration was 100 times the substrate concentration, which was 10 μM for L-phenylalanine and 14 μM for L-leucine.

[b] Conversion of substrate in the absence of inhibitor represents 100% conversion.

strate, suggesting that the enzyme is an amino acid oxidase specific for the L-stereoisomer. Enzyme production is controlled by nitrogen metabolite repression. The proposed sequence of events whereby the alpha-amino group is removed from the amino acid by the oxidase transported into cells and subsequently incorporated into glutamate and glutamine is shown in Fig. 5.

Four classes of amino acids are evident with respect to the ability to trigger deaminase production. One class, represented by L-glutamate, L-glutamine, L-asparagine, L-lysine, L-tryptophan, and L-proline, fail to elicit production. L-Ornithine and L-alanine can cause enzyme production only when all three amino acid permeases are nonfunctional. Arginine, in contrast, can elicit production in any strain containing the *pmg* mutation, which renders the general permease nonfunctional. A fourth class is composed of several neutral amino acids that promote enzyme production irrespective of permease activity. The situation is further complicated by the lack of correlation between the extent to which enzyme is produced in response to a particular amino acid and the extent to which growth is supported by that amino acid. Several factors likely underly these observations; we advance here ideas that will serve as the basis for further experimentation.

Proline, glutamic acid, glutamine, and asparagine do not trigger deaminase production, yet support growth. Proline, as an imino acid, likely gains entry by a transport system distinct from the amino acid permeases (1, 4, 10, 14, 16, 30, 40). Likewise, glutamic acid may enter cells through a separate system (24). The transport of glutamine and asparagine has not been characterized for the lower eucaryotes, and both a separate transport route and the production of catabolic enzymes specific to these amino acids could explain their utilization.

L-Lysine and L-tryptophan fail both to produce enzyme and to support growth. Since the competition studies demonstrated that these amino acids are putative substrates, we expected them to elicit deaminase. It may be that, as with histidine, a minimal level of growth must occur before measurable levels of deaminase are produced. Identification of an alternate nitrogen source that allows deaminase production in response to amino acid would allow us to separate inability to trigger deaminase production from inability to support growth.

Failure of lysine, tryptophan, and histidine to support growth may reflect their involvement in a phenomenon, termed amino acid sensitivity, whereby the organism is adversely affected by amino acids or their metabolites (33). When amino acids serve as nitrogen sources, there may be catabolic enzymes in addition to deaminases that are produced. Such a situation has been reported for *Aspergillus nidulans* in reference to L-histidine utilization (26, 27). The inhibitory product may be formed by action of the deaminase or the additional enzymes, or the latter may successfully compete with the deaminase for the amino acid substrate but fail to produce utilizable nitrogen. The addition of ammonium relieves inhibition (Fig. 2) and may well do so through preventing the production of such enzymes.

A similar situation must be considered with respect to the failure of several neutral amino acids to support growth well

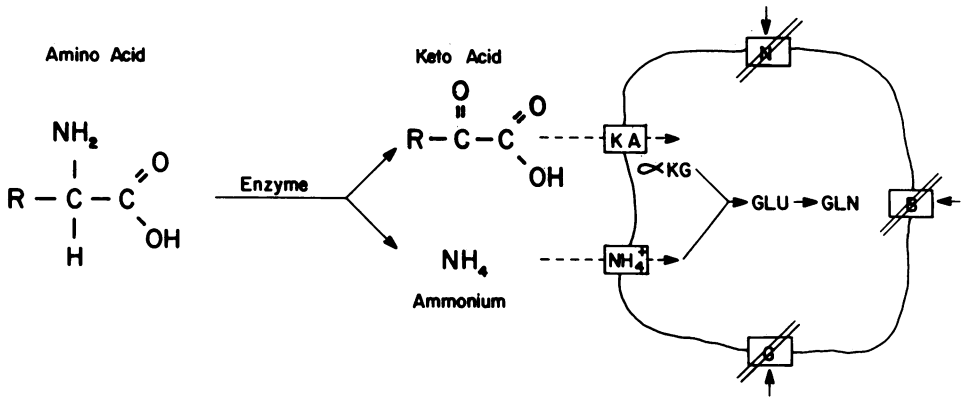

FIG. 5. Proposed model for the mechanism underlying the ability of the *pmn*; *pmb*; *pmg* strain to utilize amino acids as sole nitrogen sources. N, B, and G refer to the inactive amino acid transport systems of the *pmn*; *pmb*; *pmg* strain. α-KG, GLU, and GLN refer to α-ketoglutarate, glutamate, and glutamine, respectively, and represent the series of metabolic conversions of ammonium into organic nitrogen.

despite their ability to produce enzyme. It may be that the keto acid products are inhibitory, perhaps through interference with intermediary metabolism or with the utilization of the ammonium generated.

Three findings raise the interesting possibility that multiple L-amino acid oxidases may exist. Sikora and Marzluf have reported an oxidase produced by the wild type in response to L-amino acids and to D-methionine (31). We observed only slight enzyme production in response to D-methionine, suggesting that the activity produced by the *pmn*; *pmb*; *pmg* strain may be distinct from that previously reported. Our conditions, however, required D-methionine to serve as the sole nitrogen source, which it may be incapable of doing and cannot, therefore, elicit a detectable enzyme level. Second, we previously reported an oxidase produced by the *pmn*; *pmb*; *pmg* strain in response to FPA under nitrogen-sufficient conditions (6). In contrast to the amino acid-induced activity, the FPA-induced activity was not nitrogen regulated. The *lox* mutation eliminated production in response to FPA when ammonium was present but did not affect oxidase production in response to amino acids as nitrogen sources. These results suggest that there may be a nitrogen-independent oxidase. Third, and perhaps related to the FPA situation, is the finding that several neutral amino acids supported growth poorly and cause enzyme production, even in the wild type. The poor growth on neutral amino acids may reflect a stressful condition that elicits oxidase production analogous to the ability of biotin deficiency or protein synthesis inhibitors to trigger L-amino acid oxidase production by *N. crassa* (2, 34).

Clearly several factors must be integrated to explain the present findings. The possibility of multiple oxidases is an interesting one. It may be that *N. crassa* produces a single oxidase in response to different stress signals, analogous to the single protease produced in response to nitrogen, carbon, or sulfur starvation (3, 11). Alternatively, there may be multiple oxidases. The intracellular and extracellular oxidases may differ in a manner similar to that of the asparaginases of *S. cerevisiae* (9, 13), or each oxidase may be distinct from all other oxidases, irrespective of its intracellular or extracellular nature. Such possibilities are readily amenable to biochemical and genetic dissection, which will allow us to compare directly the oxidases produced under the various conditions that have now been reported.

ACKNOWLEDGMENTS

This research was supported by grant PCM-8304601 from the National Science Foundation.

LITERATURE CITED

1. **Arst, H. N., Jr., D. W. MacDonald, and S. A. Jones.** 1980. Regulation of proline transport in *Aspergillus nidulans*. J. Gen. Microbiol. **116**:285–294.
2. **Burton, K.** 1951. The L-amino acid oxidase of *Neurospora*. Biochem. J. **50**:258–268.
3. **Cohen, B. L., and H. Drucker.** 1977. Regulation of exocellular protease in *Neurospora crassa*: induction and repression under conditions of nitrogen starvation. Arch. Biochem. Biophys. **182**:601–613.
4. **Courchesne, W. E., and B. Magasanik.** 1983. Ammonia regulation of amino acid permeases in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **3**:672–683.
5. **DeBusk, B. G., and A. G. DeBusk.** 1965. Molecular transport in *Neurospora crassa*. I. Biochemical properties of a phenylalanine permease. Biochim. Biophys. Acta **104**:139–150.
6. **DeBusk, R. M., D. T. Brown, A. G. DeBusk, and R. D. Penderghast.** 1981. Alternate mechanism for amino acid entry into *Neurospora crassa*: extracellular deamination and subsequent keto acid transport. J. Bacteriol. **146**:163–169.
7. **DeBusk, R. M., and A. G. DeBusk.** 1980. Physiological and regulatory properties of the general amino acid transport system of *Neurospora crassa*. J. Bacteriol. **143**:188–197.
8. **DeBusk, R. M., and S. Ogilvie-Villa.** 1982. Physiological adaptation to the loss of amino acid transport ability. J. Bacteriol. **152**:545–548.
9. **Dunlop, P. C., and R. J. Roon.** 1975. L-Asparaginase of *Saccharomyces cerevisiae*: an extracellular enzyme. J. Bacteriol. **122**:1017–1024.
10. **Facklam, T. J., and G. A. Marzluf.** 1978. Nitrogen regulation of amino acid catabolism in *Neurospora crassa*. Biochem. Genet. **16**:343–354.
11. **Hanson, M. A., and G. A. Marzluf.** 1975. Control of the synthesis of a single enzyme by multiple regulatory circuits in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S.A. **72**:1240–1244.
12. **Harder, W., and L. Dijkhuizen.** 1983. Physiological responses to nutrient limitation. Annu. Rev. Microbiol. **37**:1–23.
13. **Jones, G. E.** 1977. Genetic and physiological relationships between L-asparaginase I and L-asparaginase II in *Saccharomyces cerevisiae*. J. Bacteriol. **130**:128–130.
14. **Lasko, P. F., and M. C. Brandriss.** 1981. Proline transport in *Saccharomyces cerevisiae*. J. Bacteriol. **148**:241–247.
15. **Lester, G.** 1966. Genetic control of amino acid permeability in *Neurospora crassa*. J. Bacteriol. **91**:677–684.
16. **Magana-Schwencke, N., and J. Schwencke.** 1969. A proline transport system in *Saccharomyces chevalieri*. Biochem. Biophys. Acta **173**:313–323.
17. **Magasanik, B.** 1982. Genetic control of nitrogen assimilation in bacteria. Annu. Rev. Genet. **16**:135–168.
18. **Magill, C. W., S. O. Nelson, S. M. D'Ambrosio, and G. I. Glover.** 1973. Histidine uptake in mutant strains of *Neurospora crassa* via the general transport system for amino acids. J. Bacteriol. **113**:1320–1325.
19. **Marzluf, G. A.** 1981. Regulation of nitrogen metabolism and gene expression in fungi. Microbiol. Rev. **45**:437–461.
20. **Ogilvie-Villa, S., R. M. DeBusk, and A. G. DeBusk.** 1981. Characterization of 2-aminoisobutyric acid transport in *Neurospora cassa*: a general amino acid permease-specific substrate. J. Bacteriol. **147**:944–948.
21. **Ohnishi, E., H. Macleod, and N. H. Horowitz.** 1962. Mutants of *Neurospora* deficient in D-amino acid oxidase. J. Biol. Chem. **237**:138–142.
22. **Pall, M. L.** 1969. Amino acid transport in *Neurospora crassa*. I. Properties of two amino acid transport systems. Biochim. Biophys. Acta **173**:113–127.
23. **Pall, M. L.** 1970. Amino acid transport in *Neurospora crassa*. II. Properties of a basic amino acid transport system. Biochim. Biophys. Acta **203**:139–149.
24. **Pall, M. L.** 1970. Amino acid transport in *Neurospora crassa*. III. Acidic amino acid transport. Biochim. Biophys. Acta **21**:513–520.
25. **Pall, M. L.** 1971. Amino acid transport in *Neurospora crassa*. IV. Properties and regulation of a methionine transport system. Biochim. Biophys. Acta **233**:201–214.
26. **Polkinghorne, M. A., and M. J. Hynes.** 1975. Effect of L-histidine on the catabolism of nitrogenous compounds in *Aspergillus nidulans*. J. Gen. Microbiol. **87**:185–187.
27. **Polkinghorne, M. A., and M. J. Hynes.** 1982. L-Histidine utilization in *Aspergillus nidulans*. J. Bacteriol. **149**:931–940.
28. **Rao, E. Y. T., T. K. Rao, and A. G. DeBusk.** 1975. Isolation and characterization of a mutant of *Neurospora crassa* deficient in general amino acid permease activity. Biochim. Biophys. Acta **413**:45–51.
29. **Roess, W. B., and A. G. DeBusk.** 1968. Properties of a basic amino acid permease in *Neurospora crassa*. J. Gen. Microbiol. **52**:421–432.
30. **Schwencke, J., and N. Magana-Schwencke.** 1969. Derepression of a proline transport system in *Saccharomyces chevalieri* by nitrogen starvation. Biochim. Biophys. Acta **173**:302–312.
31. **Sikora, L., and G. A. Marzluf.** 1982. Regulation of L-amino acid

oxidase and of D-amino acid oxidase in *Neurospora crassa*. Mol. Gen. Genet. **186**:33–39.
32. **Sikora, L. A., and G. A. Marzluf.** 1982. Regulation of L-phenylalanine ammonia-lyase by L-phenylalanine and nitrogen in *Neurospora crassa*. J. Bacteriol. **150**:1287–1291.
33. **Sumrada, R., and T. Cooper.** 1976. Basic amino acid inhibition of growth in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. **68**:598–602.
34. **Thayer, P. S., and N. H. Horowitz.** 1951. The L-amino acid oxidase of *Neurospora*. J. Biol. Chem. **192**:755–767.
35. **Vaca, G., and J. Mora.** 1977. Nitrogen regulation of arginase in *Neurospora crassa*. J. Bacteriol. **131**:719–725.
36. **Vogel, H. J.** 1964. Distribution of lysine pathways among fungi: evolutionary implications. Am. Nat. **48**:435–446.
37. **Wolfinbarger, L., Jr., and A. G. DeBusk.** 1971. Molecular transport. I. *In vivo* studies of transport mutants on *Neurospora crassa* with altered competition patterns. Arch. Biochem. Biophys. **144**:503–511.
38. **Wolfinbarger, L., Jr., H. H. Jervis, and A. G. DeBusk.** 1971. Active transport of L-aspartic acid in *Neurospora crassa*. Biochim. Biophys. Acta. **249**:63–68.
39. **Woodward, J. R., and V. P. Cirillo.** 1977. Amino acid transport and metabolism in nitrogen-starved cells of *Saccharomyces cerevisiae*. J. Bacteriol. **130**:714–723.
40. **Zalokar, M.** 1961. Kinetics of amino acid uptake and protein synthesis in *Neurospora*. Biochim. Biophys. Acta **46**:423–432.