The Effect of Lack of a Carbon Source on Nitrate-reductase Activity in *Aspergillus nidulans*

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Control of nitrate-reductase levels has been extensively studied in *Aspergillus nidulans* (Pateman & Cove, 1967; Cove & Pateman, 1969). During studies on the interaction between regulation of carbon metabolism and regulation of nitrogen metabolism (Hynes, 1972) preliminary evidence has been obtained indicating that a gene involved in the interaction affects growth of *A. nidulans* on nitrate and nitrite (M. J. Hynes, unpublished). A study of the effects of carbon starvation on nitrate-reductase levels has therefore been carried out.

Mycelium of the wild-type strain, *biA*-1, was grown under inducing conditions and then transferred to various media and incubated further. Mycelial samples of 20 to 30 ml were harvested at intervals and assayed for nitrate-reductase activity by the method of Cove (1966) with the modified control of Lewis & Fincham (1970).

As shown in Fig. 1 (*a, b*), nitrate-reductase activity rapidly disappeared in medium in which further synthesis of the enzyme was not possible or in medium lacking a carbon source. Absence of inducer and repression by ammonium caused rapid loss of activity as has been shown previously for *Aspergillus nidulans* (Cove, 1966), *Neurospora crassa* (Subramanian & Sorger, 1972) and *Ustilago maydis* (Lewis & Fincham, 1970). In the latter organisms protein-synthesis inhibitors prevent this inactivation. The results presented here indicated that cycloheximide, at 10 µg/ml, only slightly reduced the rate of enzyme loss and, in fact, cycloheximide itself caused loss of nitrate-reductase activity. A lower concentration of cycloheximide (1 µg/ml) had a similar effect. Methylammonium chloride, which does not serve as a nitrogen source for the strain *mau* A-2 (Arst & Cove, 1969) caused rapid loss of activity in this strain (results not shown).

An even greater rate of enzyme disappearance was found when mycelium was starved for carbon. The rates of inactivation were similar when brought about by carbon starvation in the presence of inducer or inducer plus ammonium, or in the absence of inducer. This suggests that there is only one mechanism of inactivation operating under the various conditions.

The data in Fig. 1 (*c*) show that inactivation of nitrate reductase was also found in the mutant *nir* A⁻-1, which produces nitrate reductase in the absence of inducer (Pateman & Cove, 1967). This strain was grown in glucose-minimal medium with 5 mM-urea as sole nitrogen source before transfer to various media. Both ammonium repression and carbon starvation resulted in loss of activity. Transfer to medium lacking a nitrogen source resulted in an increase in activity, presumably owing to relief of repression by ammonium produced during growth on urea. This indicates that starvation for nitrogen in itself does not cause inactivation.

Rapid loss of enzyme activity with starvation for a carbon source is not a unique property of nitrate reductase. Unpublished experiments have shown that NADP-glutamate dehydrogenase and glucose-6-phosphate dehydrogenase activities rapidly decrease in the absence of

Fig. 1. Disappearance of nitrate reductase activity under various growth conditions. (a, b) Wild-type pre-induced with 10 mM-NaNO₃; (a) glucose-minimal medium; (b) minimal medium lacking a carbon source; (c) *nirA*⁻¹ pre-grown on glucose-5 mM-urea medium. ●—●, no nitrogen source; △—△, 10 mM-NaNO₃; ▲—▲, 10 mM-NaNO₃+20 mM-NH₄⁺; ○—○, 10 mM-NaNO₃+cycloheximide (10 µg/ml); □—□, 10 mM-NaNO₃+20 mM-NH₄⁺+cycloheximide (10 µg/ml); ●—●, glucose-minimal medium with no nitrogen source; ▲—▲, glucose-minimal medium+10 mM-NaNO₃+20 mM-NH₄⁺; ○—○, minimal medium lacking a carbon source+10 mM-NaNO₃.

a carbon source, but for these two enzymes cycloheximide prevents the inactivation. It is not known if this loss of enzyme activity is due to protease action on the enzymes. An attempt to demonstrate *in vitro* inactivation of nitrate reductase and glutamate dehydrogenase by mixing crude mycelial extracts and incubating was not successful. However, this experiment did show that it was unlikely that accumulation of an inhibitor of the enzymes during carbon starvation could account for the results.

It has been shown previously that *Aspergillus nidulans* produces increased amounts of an acetamidase enzyme upon carbon starvation even in the absence of inducer (Hynes, 1970, 1972). Pre-induced β -galactosidase activity is not rapidly lost from carbon-starved mycelium, nor does the activity of this enzyme increase in medium lacking a carbon source in the absence of an inducer (M. J. Hynes, unpublished). Therefore it seems that various enzymes show different responses to carbon starvation and different absolute requirements for the presence of inducer. In the case of nitrate reductase there is an absolute requirement for inducer and for the presence of a carbon source. If inactivation of enzymes upon carbon starvation involves protease digestion of the enzyme protein, then this would provide an additional source of carbon and nitrogen for the organism.

Cove (1972*a, b*) has shown that nitrate and chlorate inhibit the utilization of various nitrogen sources and that the nitrate-reductase enzyme protein is required for this inhibition. It has been found that 10 mM-sodium nitrate inhibits the utilization of acetamide as a nitrogen source in the strain *niiA*-4 which is unable to utilize nitrate owing to lack of nitrite-reductase activity (Pateman & Cove, 1967). Potassium chlorate (150 mM) inhibits utilization of acetamide as sole nitrogen source by both wild-type and *niiA*-4 strains. Various mutants with altered nitrate reductase are resistant to these inhibitions. In contrast to these effects on the utilization of acetamide as a nitrogen source, nitrate and chlorate do not inhibit the utilization of acetamide as sole carbon and nitrogen source. The effect of the absence of a carbon source on nitrate-reductase activity reported here may be of importance in explaining these observations and further studies are required.

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