Activity and Regulation of an Acidic Amino Acid Permease of *Aspergillus nidulans*

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An acidic amino acid permease is formed during germination of *Aspergillus nidulans* conidia. This permease is inhibited by sodium azide (95% at 10 mM), is inactive at 2°C and exhibits both pH and temperature optima. Uptake of glutamate occurs when the intracellular glutamate concentration is 100 times the extracellular concentration. Basic and neutral amino acids do not competitively inhibit the permease. L-Aspartate and L-cysteic acid are competitive inhibitors of glutamate uptake, and conversely glutamate is a competitive inhibitor of aspartate uptake. The K_m for glutamate at 30°C is 180 μ M; the K_i for glutamate inhibition of aspartate uptake is 140 μ M. There is similar agreement between the values for aspartate (K_m , 100 μ M; K_i for inhibition of glutamate uptake, 140 μ M). The K_m for glutamate is the same both before and some hours after germ-tube emergence. Mycelium germinated on glutamate as sole nitrogen source has the same K_m for glutamate as mycelium germinated on ammonia.

Aspergillus conidia take up glutamate at very low rates. Permease activity increases 100-fold during germination when one of the following compounds is the sole nitrogen source: L-glutamate, L-aspartate, L-cysteic acid, glycine, L-lysine. The increase is 50-fold with nitrate as sole nitrogen source, but less than eightfold with ammonia, methylamine, urea or asparagine. The increase in permease activity resulting from germination in a glutamate medium is prevented by the simultaneous presence of ammonia. This effect of ammonia is less marked in a mutant that is resistant to methylamine; some of the enzymes of this mutant are resistant to repression by ammonia (Arst & Cove, 1969). When conidia germinated with ammonia as nitrogen source are transferred to a glutamate medium the permease activity increases tenfold within 4 h. This change in activity is prevented by ammonia (25 mM) or cycloheximide. These results suggest that formation of the permease is regulated by a mechanism involving repression by ammonia, or by a product of ammonia metabolism.

Further, there is some evidence that ammonia, or a metabolite of ammonia, inhibits the permease. Preincubation for 30 min with ammonia (25 mM) leads to about 40% inhibition of the rate of glutamate uptake, but no inhibition occurs when ammonia is added 1 min before glutamate (0.1 mM).

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A Cytochrome-Deficient Mutant of *Pseudomonas* AM1

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Two mutants of *Pseudomonas* AM1 have been isolated that have lost the ability to oxidize methanol and ethanol. Except for these alcohols, mutant PCT-29 grows on the same substrates as wild-type *Pseudomonas* AM1 and is able to oxidize formaldehyde, formate, methylamine, succinate and malate. Mutant PCT-76 is superficially similar, except that methylamine does not support growth and whole cells do not oxidize this substrate. Mutant PCT-76 may be a double mutant; its properties with respect to methylamine are therefore not further discussed in this communication.

Ultrasonic extracts of mutant PCT-29 do not have the alcohol dehydrogenase described by Anthony & Zatman (1965), showing that this enzyme is essential for growth of *Pseudomonas* AM1 both on methanol and on ethanol.

Ultrasonic extracts of mutant PCT-76, however, have wild-type activities of the alcohol dehydrogenase. The failure of this mutant to oxidize alcohols can be explained by the absence of cytochrome *c*, which is the only soluble cytochrome found in wild-type *Pseudomonas* AM1 (Anthony, 1970). Ultrasonic extracts of mutant PCT-76 contain no soluble cytochromes and, further, no cytochrome *c* can be observed in whole cells of this mutant.

Membrane fragments from mutant PCT-76 contain all the cytochromes and all the carotenoid pigment of the mutant, and appear to be identical with membrane fragments from the wild-type organisms. Results obtained with pigment mutants of *Pseudomonas* AM1 indicate that the carotenoid pigments are not involved in electron transport in this organism. The *a*-type cytochrome, which is found together with *b*-type cytochrome in membrane fragments from mutant PCT-76 and wild-type organisms, is probably the cyanide-sensitive component involved in the oxidation of all substrates by *Pseudomonas* AM1.

The results presented in this communication demonstrate an unexpected complexity in the electron-transport system in *Pseudomonas* AM1. The cytochrome *c* of this organism can interact with the systems for electron transport from all substrates that can be oxidized (Anthony, 1970).

The absence of the soluble cytochrome *c*, however, does not prevent the oxidation of formaldehyde, formate, succinate or malate.

It is concluded from the characteristics of mutant PCT-76 that the soluble cytochrome *c* of *Pseudomonas* AM1 is not involved in the oxidation of the majority of substrates, but that it is essential for the oxidation of methanol and ethanol.

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The Involvement of Glycollate in the Pathway for Ethanol Assimilation in *Pseudomonas* AM1

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In *Pseudomonas* AM1 C_1 compounds are assimilated by the serine pathway (Large, Peel & Quayle, 1961); none of the enzymes of this pathway is involved in known routes of ethanol metabolism. A mutant (PCT-48) of *Pseudomonas* AM1, unable to grow on C_1 compounds or ethanol, has been isolated. This suggests a novel pathway for ethanol metabolism that has one or more enzymes common to the pathway for the assimilation of C_1 compounds.

Mutant PCT-48 has all of the known enzymes required for C_1 -unit assimilation. Further, wild-type ethanol-grown organisms have very low activities of serine-glyoxylate aminotransferase, an inducible enzyme of the serine pathway, indicating that ethanol is not metabolized by this route. Malate synthetase is the only enzyme of the glyoxylate by-pass found in ethanol-grown *Pseudomonas* AM1. The trace amount of the key enzyme isocitrate lyase (specific activity less than $0.1 \mu\text{mol/h per mg}$) is insufficient to account for the growth rate on ethanol (mean generation time 7h).

Acetate is likely to be an intermediate in ethanol assimilation; ethanol-grown organisms contain enzymes that oxidize ethanol to acetate as well as the enzymes of the tricarboxylic acid cycle. The results of short-term incubation of ethanol-grown *Pseudomonas* AM1 with $[U-^{14}\text{C}]$ acetate indicate that malate and glycollate are early intermediates in acetate metabolism. Very little label was present at early times in succinate, fumarate, citrate, glycine or serine.

Substrate amounts of glycollate allow growth of mutant PCT-48 on ethanol or acetate. This supports the suggestion that glycollate is an intermediate in ethanol and acetate assimilation. Further,

the hydroxypyruvate reductase mutant 20-BL (supplied by Professor J. R. Quayle) does not grow on ethanol even if glycollate is present. This mutant grows on ethanol plus glyoxylate indicating that the oxidation of glycollate to glyoxylate is involved in ethanol assimilation. Malate synthetase could provide a mechanism for malate formation from glyoxylate and acetate.

A possible sequence of intermediates in the assimilation of ethanol is as follows: ethanol, acetaldehyde, acetate (or derivative), glycollate, glyoxylate, malate.

Mutant PCT-48 has possibly lost an enzyme involved in the formation of glycollate from an acetate derivative. This enzyme may also be important for growth on C_1 compounds, because mutant PCT-48 can grow on methanol only in the presence of glycollate or glyoxylate but not acetate.

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In Search of Lactate Dehydrogenase from *Bacillus stearothermophilus*

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In the course of a multienzyme preparation from *Bacillus stearothermophilus* (Suzuki & Harris, 1971) by using fractionation with ammonium sulphate followed by column chromatography on DEAE-cellulose and hydroxyapatite, what appeared to be a pure protein possessing both lactate dehydrogenase and alcohol dehydrogenase activities was isolated. The elution profiles of the two activities from both columns coincided exactly and the purified enzyme also appeared to be homogeneous when examined by electrophoresis on gelatinized cellulose acetate (Cellologel) at different pH values, with and without 8M-urea, as well as on polyacrylamide gel in 0.1% sodium dodecyl sulphate. It also gave a single symmetrical peak in the analytical ultracentrifuge ($s_{20,w}$ 6.0S) and the molecular weight was estimated to be $135000 \pm 3\%$ by the sedimentation-equilibrium method. The molecular weight in 0.1% sodium dodecyl sulphate was estimated to be approx. 34000, and from these data it appeared that we had succeeded in isolating a tetrameric enzyme possessing both lactate dehydrogenase and alcohol dehydrogenase activities.

Subsequently, however, while attempting to purify phosphofructokinase from the same cell extracts it was found that this activity was also associated with the lactate dehydrogenase and