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Ammonium transport using ^{14}C-methylamine as substrate

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Abstract

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Neurospora crassa wild type, Tatum a (5Y4fg a) conidia, possesses an active transport system for ammonium which can be characterized using ^{14}C-methylamine as an ammonium analog. In nitrogen-deficient medium (Vogel's minimal medium with NH_4NC_3 omitted but containing 1% glucose), the initial rate of uptake of ^{14}C-methylamine shows Michaelis-Menten saturation kinetics with a V_{max} of about 15 μmoles/g/min and a K_m of about 1.6×10^{-5} molar. The determination of Michaelis-Menten constants was done over a range of 2×10^{-4} M to 5×10^{-7} M methylamine. The velocities (Table 1), were determined by initial rates determined from 1, 2, 3 and 4 minutes sample points with conidia that had been preincubated in nitrogen-deficient medium containing 1% glucose for 2 hours prior to the addition of ^{14}C-methylamine.

Table 1

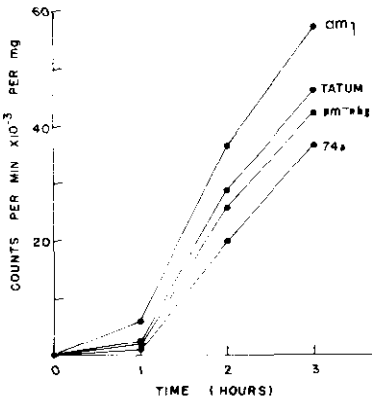
Methylamine transport rates at various substrate concentrations

Substrate conc. (molar)	2×10^{-4}	1×10^{-4}	5×10^{-5}	2×10^{-6}	1×10^{-6}	5×10^{-7}
Velocity μmoles/g/min	14.89	12.65	10.95	1.66	0.85	0.46

Transport rate greatly increases with nitrogen starvation, with or without glucose present. However, if glucose is absent, there is no saturation of transport even up to 2 mM methylamine. Inhibition kinetics show that NH_4Cl competitively inhibits (K_i - 4.7 μmolar) ^{14}C-methylamine uptake, showing that methylamine is a suitable ammonium analog. Transport at pH 5.6 in nitrogen-deficient medium shows that prior development of transport capability is optimal at pH 3 to 4. However, if developed at pH 5.6 in nitrogen deficient medium containing glucose, subsequent transport increases with a pH increase up to pH 9. In addition to protein synthesis (as exemplified by cycloheximide inhibition), energy is required to maintain or produce uptake capability as demonstrated by sodium azide and 2,4-dinitrophenol sensitivity. Furthermore, transport is temperature dependent with a Q_{10} of 1.9 between 20 and 30° C.

Accumulation assays (in nitrogen-deficient medium containing glucose) using ^{14}C-methylamine, were performed with Tatum a, 74-OR8-1a, am1 (FGSC #521), and pmg;mtr;bat (Pm^-nbg) (FGSC #2606) (deficient in neutral, basic, and general amino acid transport; Rao and De Busk 1975 B.B.A. 413:45; 1976 Neurospora Newsl. 22: 12-13) and can be compared in Figure 1. It is interesting to note that the absence of the NADP-linked glutamate dehydrogenase does not prevent methylamine (and presumably ammonium) transport since am1, which lacks this enzyme, is capable of ^{14}C-methylamine transport. It has been reported (Dubois, Grenson and Wiame 1973 B.B.R.C. 50: 967) that glutamate dehydrogenaseless mutants in yeast are derepressed in the presence of ammonium for ammonium repressible activities. The data implies that such phenomena in Neurospora may not be due to lack of transport of ammonium since am1 transports methylamine even better than wild type. Also, pmg;mtr;bat (Pm^-nbg) transports ^{14}C-methylamine almost as well as the parental strain, Tatum a, which shows the separate identity of amino acid and ammonium transport systems.

Figure 1



Conidial transport of ^{14}C-methylamine at 25° C by several strains of Neurospora crassa in nitrogen deficient medium containing 1% glucose. The isotope concentration was 1×10^{-4} M at a specific activity of 0.1 μCi/0.1 μmole/ml.

A three day growth test at 25° C revealed that methylamine cannot serve as a carbon or nitrogen source for Neurospora. Instead, methylamine, at 10 mM, is toxic to Neurospora on a nitrate medium (Vogel's medium N substituting KNO_3 for NH_4NO_3) but not on an ammonium medium (Vogel's medium N with NH_4Cl instead of NH_4NO_3), thus supporting the hypothesis that methylamine and ammonium ions utilize the same transport system.

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