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"Development and Characterization of Guanine Transport"

Mutant in *Neurospora crassa*

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ABSTRACT

Initial attempts to find mutant strains incapable of transporting guanine into the cells yielded no mutants. Further studies on the transport of guanine into N. crassa cells revealed a second guanine uptake system which also bound hypoxanthine but appeared not to transport it. This guanine-specific system appeared to be energy-dependent, in that it was inhibited by sodium azide but was not sensitive to 2,4-dinitrophenol.

INTRODUCTION

Cells are highly selective in determining which compounds may cross the cell membrane and enter the cell. This selectivity is due to transport systems present in the cell membrane. These transport systems (usually proteins) recognize specific chemicals, bind them and transport them into the cell. Transport processes are most obvious when they are absent, i.e., when a mutant cell is unable to take up a certain chemical which normal cells transport. Thus, the study of transport systems is greatly facilitated by studying mutant cells which lack them.

Purine bases are thought to be transported into many cells but the mechanism of transport is as yet unknown in many organisms. Guanine, a purine base, is probably transported into cells of the fungus, Neurospora crassa because preliminary experiments suggest that guanine can be used as the sole source of nitrogen for the cell. Mutants of Neurospora which lack the ability to transport guanine may give a clue as to:

a) what other compounds the same transport system takes up,

b) the capacity of the transport system under different environmental conditions, and

c) the relationship to internal metabolic reactions.

EXPERIMENTAL PROCEDURE

1. Selection of mutants

Mutations were induced using a filtration enrichment procedure, where organisms were first exposed to a mutagenic agent, UV light. They were then added to a selective media, in this case, one where guanine was the sole nitrogen source. Colonies which grow were filtered off. Those organisms remaining were assumed to be mutants and were then plated onto minimal media plus a nitrogen source other than guanine.

2. Transport studies

Two ml of germinated conidia (4×10^6/ml) are added to 2 ml of a reaction mixture containing a known concentration of labeled compound (and competitor if inhibition is being tested). The mixture is incubated for a time period within the range known to give linear uptake, and the contents are collected on a glass fiber filter. The filters are washed and dried thoroughly for radioactivity counting by liquid scintillation methods.

RESULTS AND DISCUSSION

No guanine transport mutants were obtained from three different mutation runs using the filtration enrichment techniques described in the experimental procedure section. Investigation of the transport of guanine into cells of the ad-8 strain suggested that in wild type strains guanine must be transported by two different transport systems. If there are two transport systems capable of mediating guanine uptake, then that could explain why no guanine transport mutants were found.

Further studies on guanine transport clearly indicate that there is a guanine-specific transport system besides the general base transport system described previously (1). In the ad-8 strain (Figure 1) ^{14}C guanine is transported into cells by an energy-dependent, mediated process. The transport of guanine was inhibited by sodium azide, which indicates that the electron transport system is involved, but not by 2.4 dinitrophenol. Results of experiments designed to show the specificity of this system in ad-8 (Fig. 2) show that transport of guanine is inhibited only by hypoxanthine which is not transported. This strongly suggests that hypoxanthine participates in non-productive binding with the guanine-specific transport system.

LITERATURE CITED

1. Magill, J. M. and C. W. Magill. 1975. Purine base transport in Neurospora crassa. J. Bacteriol. 124:149-154.

Fig. 1. Transport of ^{14}C guanine into germinated conidia of ad-8 strain

- ● ^{14}C guanine alone
- △ ^{14}C guanine + 1mM 2,4 DNP
- □ ^{14}C guanine + 1mM Na azide

Fig. 2. Transport of ^{14}C guanine into germinated conidia of ad-8 strain.

- ● ^{14}C guanine alone
- ○ ^{14}C guanine + 1mM hypox
- □ ^{14}C guanine + 1mM unlabelled guanine

Fig 1 Energy dependence of Guanine transport

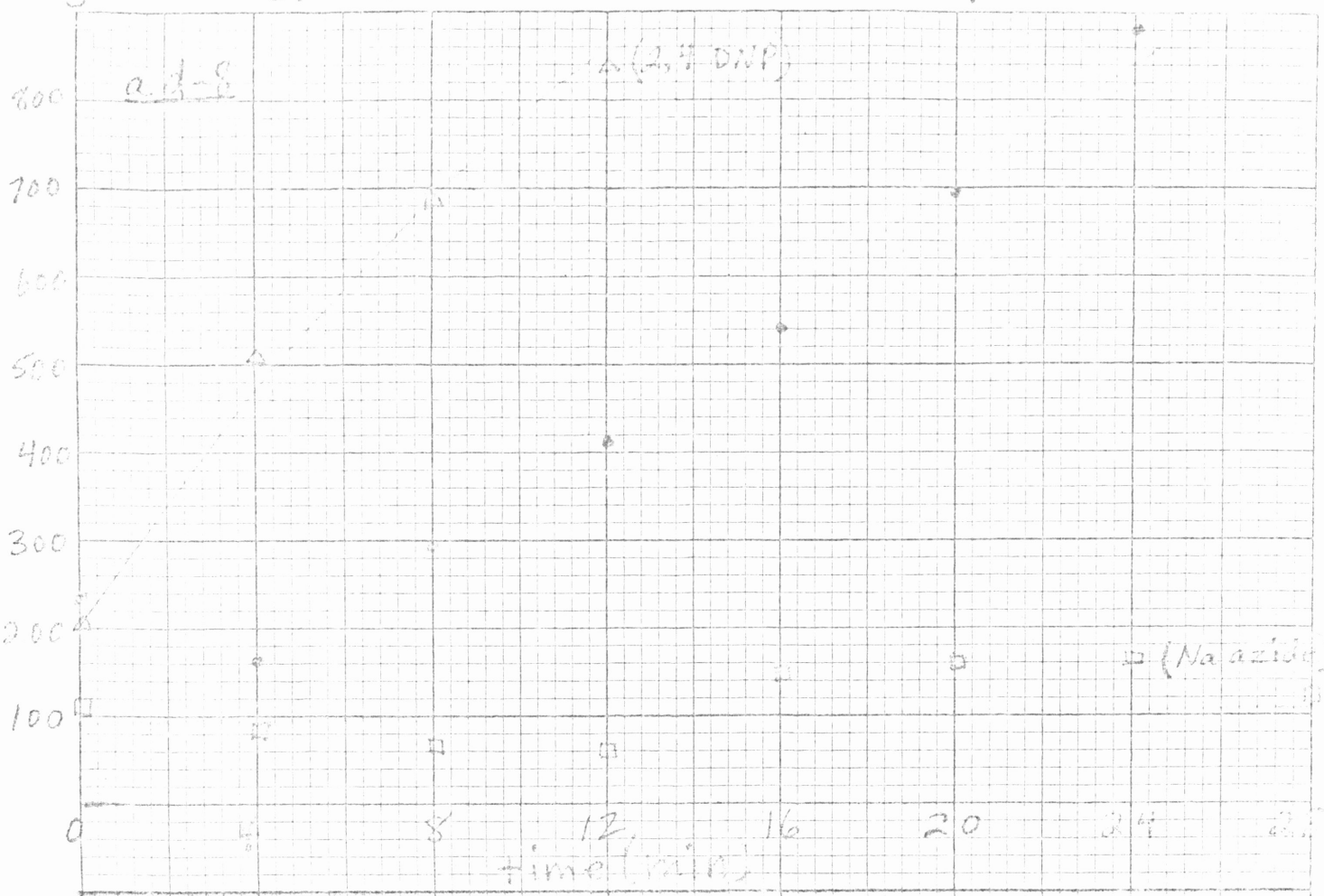

Fig 2 Specificity of Guanine transport