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Tyrosine transport in Neurospora

Abstract

Tyrosine transport in Neurospora

Brescia, V. T. Tymsine transport in *Neurospora*.

Uptake of tyrosine by *Neurospora* conidia was studied using ^{14}C tyrosine in the manner described by DeBusk and DeBusk (1965 *Biochim. Biophys. Acta* 104: 139) for phenylalanine. Conidial suspensions

which consistently gave 0.19 - 0.28 mg dry weight of conidia per 5 ml sample were prepared by adjusting OD_{397} to 0.9-0.95 (B and L Spectronic 20). The usual conditions were a temperature of 30°C and tyrosine concentration of $1\ \mu\text{mole}$ per 25 ml ($4 \times 10^{-5}\text{ M}$) reaction mixture (Vogel's minimal + cells). The optimum temperature was later found to be between $31-33^\circ\text{C}$ and the pH optimum 5.8.

Incubation at 45°C for 20 minutes did not inactivate the transport system - as little as 2 minutes at 50° did temporarily inactivate (uptake less than 70% of control at 20 minutes). Recovery occurred in cells held at 30° for 30 minutes following 50° heat inactivation. Concentrations from $0.2\ \mu\text{mole}/25\text{ml}$ to $3.2\ \mu\text{mole}/25\text{ml}$ gave increasing initial rates of uptake; no increase was observed above $5\ \mu\text{mole}/25\text{ml}$. A reciprocal plot of initial uptake vs tymsine concentration (Lineweaver-Burke) gave a straight line. By extrapolation, the K_m was estimated at $1.2-1.8 \times 10^{-4}\text{ M}$ in three experiments. After 50 minutes uptake, the amount of label chromatographically identical with tyrosine that can be extracted with 5% TCA at room temperature in 10 minutes is at least 30 x the external concentration.

Glucose (final conc. 1%) added to an actively transporting system will inhibit further transport within 6 minutes and will continue inhibiting for at least 15 minutes, after which transport is resumed, apparently at the same rate. Sodium azide and 2,4-dinitrophenol at 10^{-3} M restrict transport to about 10% of the control. With azide, at least the inhibition is almost instantaneous. A variety of compounds were tested at concentrations 25 x that of tymsine for their effects on uptake of ^{14}C L-tyrosine at a concentration of $4 \times 10^{-5}\text{ M}$. Shikimic acid and para-hydroxyphenylpyruvate, among others, had no effect whereas L-tryptophan and L-phenylalanine reduced uptake to 20% or less of control. Since all of the &we-mentioned compounds can supplement appropriate mutants, they must be capable of entering the cell. Therefore, the lack of effect of shikimic acid and para-hydroxyphenylpyruvate must reflect a stereospecificity of the tyrosine transport system. This is further demonstrated by the fact that D-tyrosine reduces uptake to 87% of control, whereas an equivalent amount of ^{12}C L-tyrosine reduces it to 25% of control. ■ ■ ■ Department of Biological Science, Florida State University, Tallahassee, Florida 32306.