Cytochrome spectra of cytoplasmic mutants

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Recommended Citation
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Abstract
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This research note is available in Fungal Genetics Reports: http://newprairiepress.org/fgr/vol13/iss1/2
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$_{370}$ to 0.9-0.95 (B and L Spectronic 20). The usual conditions were a temperature of 30°C and tyrosine concentration of 1 $\mu$mol per 25 ml (4 x $10^{-5}$ M) reaction mixture (Vogel’s minimal + cells). The optimum temperature was later found to be between 31-33°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°C did temporarily inactivate (uptake less than 70% of control at 20 minutes). Recovery occurred in cells held at 30°C for 30 minutes following 50°C heat inactivation. Concentrations from 0.2 $\mu$mol/25 ml to 3.2 $\mu$mol/25 ml gave increasing initial rates of uptake; no increase was observed above 5 $\mu$mol/25 ml. A reciprocal plot of initial uptake vs tymsine concentration (Lineweaver-Burke) gave a straight line. By extrapolation, the Km was estimated at 1.2-1.8 x 10^{-4}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% of 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 x 10^{-5} 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 above-mentioned compounds 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.


Definitive studies on the absorption spectra of the Neurospora cytoplasmic mutants (polk) and (mi-3) were originally performed by Mitchell et al. (1953 Proc. Natl. Acad. Sci. U. S. 39: 606) and Tissieres and Mitchell (1954 J. Biol. Chem. 206: 241). Their studies were done with a hand spectroscope on mycelial pads and crude mitochondrial suspensions. The present work essentially repeats their experiments, but derives the cytochrome spectra spectrophotometrically from disrupted mitochondrial preparations. Other maternally-inherited mutants are also examined.

Mitochondria were prepared by a method similar to that used by Luck (1965 J. Cell Biol. 24: 445). Mycelium was grown in liquid shake cultures at 30°C and harvested in the exponential growth phase. After grinding with sand in 0.01 M Tris buffer containing 0.001 M EDTA (adjusted to pH 7.3) and 0.44 M sucrose, cell debris was removed by 20 minute centrifugations at 1000 x g. Mitochondria were spun down in a 30 minute centrifugation at 20,000 x g and washed once in the buffered sucrose. The resulting crude mitochondrial pellets were disrupted by sonicication and the solutions cleared by adding sodium deoxycholate to a concentration of 2%. Spectra were read in a Cary 16 spectrophotometer, a few crystals of sodium dithionite being added to the sample cuvette to reduce the cytochromes. All the spectrums were read from solutions containing 10-20 mg/ml of protein, estimated by the Folin test.

It has been found that the cytoplasmic mutants tested fall into two groups on the basis of their spectra. The first group, consisting of [polk] (3627-Z) (FGSC#384), suppressed [polk] (polpolk) (FGSC#386 and 385), [SG-3] (no isolation #, FGSC#1452), a UV-induced stopper strain ( [typ] 30a-4, FGSC#1573 McDougall and Pittenger 1966 Genetics 54: 551), and two stopper strains spontaneously arisen in separate continuous growth tubes ( [typ-A] 40-4, and [typ-B] 17-2a-1, Bertmnd and Pittenger 1968, in preparation). All of these strains show identical mutant spectra of the type shown in Figure 1. The notable features are an absence of cytochromes a (610 mp) and b (560 mp), and a very marked a-cytochrome c peak (550 mp). The published data of Diaacumakos et al. (1965 J. Cell Biol. 26: 427) reveal that [abn-1] also belongs to this group. A typical wild type spectrum is shown in Figure 2. r o r [mi-1] FGSC#343 exhibited a wild type spectrum.

The second group consists of the [mi] strains, [mi-2] to [mi-8] (mi-2R to mi-7R and mi-8R) (FGSC#s 1233, 383, 1234, 1235, 1236, 1237, 1238), and a typical spectrum is shown in Figure 3. Cytochrome a is again absent, cytochrome b is present in wild type amounts, and cytochrome c is again in excess. In the work of Mitchell et al., a strong band was observed at 590 mp in [mi-31] and labelled cytochrome a; this has never been observed in our experiments. The [mi] strains [mi-2] to [mi-8] are in fact probably replicates of the same mutant (M. B. Mitchell, personal communication).
On occasion (mi-31 A (FGSC#383) has shown a spectrum closer to that of wild type. The cause of this apparent reversion is not known, but it has also been observed by other workers (Grindle and Woodward 1967 Neurospora News. 12: 9).

The two nuclear genes known to affect cytochrome content in Neurospora have also been examined: cyt-2 (C117) (FGSC#339) is shown in Figure 4, and is similar to the spectrum obtained for this strain by Mitchell et al., in that cytochromes a and c are both absent, but differs in that no cytochrome e is detected at 553 mu. cyt-1 (C115) (FGSC#555) shows on essentially wild type spectrum. Tissieres and Mitchell (1954) have, however, indicated that C115 is particularly prone to suppression, so it must be concluded that this is the case in the culture tested. cyt-1 (C115) (FGSC#1217) was not tested.

The β-peaks of cytochromes c (520 mu) and b (530 mu) are seen to vary in accordance with the α-peaks. From the curves it is possible to calculate the absolute counts of cytochrome present. However, it is apparent that the relative amounts are more useful in diagnosing mutant types. The fact that the above spectra are in the main port very similar to the mycelial spectra produced by Mitchell et al. is indicative that the whole-cell cytochrome content reflects, to a large degree, the mitochondrial bound complement, which in turn is presumably dependent on the basic genetic lesion responsible for the maternal inheritance of the metabolic defects. The gene f does not suppress [mi-31], and the cytoplasmic mutants in the first group described above were induced in a variety of nuclear backgrounds. Thus it seems reasonably certain that the groups represented by [poky] and [mi-3] reflect truly different types of genetic lesions, in two regions of either one or two mitochondrial genes, (68; 31; 47; 72) concerned, perhaps, with structural protein and are not nuclear modifications of each other.

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NOMENCLATURE


The isolation of urease defective mutants was reported previously (Kolmark 1965 Neurospora News. 8: 6). The symbol ur was used in this first report for permanent use it appears that the symbol ure would be the preferential choice for the reasons explained below.

The symbol ur is sometimes used for umcill requirement, e.g., in yeast (von Borstel (ed.) 1963 Microbial Genet Bull., Suppl. to No. 19). On the other hand, it is proposed in a list of symbols for mutants in bacterial strains that uracil requirement be designated by ura (Demerec 1963 Microbial Genet Bull. 19: 30). In this article it is also recommended that tri-letter abbreviations be used as mutant symbols. In Streptomyces coelicolor ura is used for uracil requirement while ure is used for urease defectiveness (Hopwood 1965 Genet. Res. 6: 248). Obviously, this provides a clear distinction when both of these mutant characters occur in the same organism.

Since the first report of urease mutants in Neurospora crassa, mentioned above, it has been established that there are two separate loci for this character (Kolmark 1968, this issue of Neurospora News.). These loci are referred to as we-1 and we-2. The original isolation numbers are for future use maintained as allelic designations, (2) and (47), respectively.

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