Alternate forms of cytochrome c in the mi-1 (poky) mutant of Neurospora crassa

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Abstract
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Arginine is actively transported into *Neurospora crassa* (74A) conidio by a constitutive, stereospecific permease system showing characteristic Michaelis-Menten kinetics and a Km of $2 \times 10^{-5}$ M. The Process is temperature-dependent with an optimum at 35°C. A pH optimum occurs at 5.6. The amino acid is trans- ported a concentration gradient, resulting in an intracellular arginine concentration some 1450-fold greater than that of the external medium. The transport process is energy-dependent as shown by its complete inhibition by NaN₃ and DNP. No influx of previously accumulated arginine occurs either in the absence of external substrate or in the presence of energy uncoupling agents.

Stereospecificity of the transport system is indicated by transport competition studies with a number of amino acids. All L-isomers tested showed varying degrees of inhibition except proline which is characterized by a poor inhibitor for all permease systems studied. D-arginine, at concentrations 5-fold that of L-arginine, does not inhibit the transport of the L-isomer. The basic amino acids lysine and ornithine were very effective inhibitors, while glutamic acid was a poor inhibitor. The reduction in arginine transport at various inhibitor-to-arginine ratios is summarized in Table 1.

Simultaneous transport of pairs of amino acids was studied in order to further evaluate specificity and possible overlap of transport families. In all cases, the concentration of each amino acid was sufficiently high to saturate the permease enzyme(s) (rate independent of concentration). When lysine-$\text{C}^{14}$ and arginine-$\text{C}^{14}$ were simultaneously transported, the resulting rate was the average of their independent rates. This would indicate that arginine and lysine are transported by a common permease system. Very different results were obtained when phenylalanine-$\text{C}^{14}$ and arginine-$\text{Cl}^{4}$ were simultaneously transported. The initial rate of $\text{C}^{14}$ transport was 80% of the sum of the independent rates for the individual amino acids. After 30 minutes the rate was nearly equal to the rate of arginine transport alone. This would suggest the existence of separate permeases for phenylalanine and arginine.

The inhibition of arginine transport by phenylalanine and other amino acids might be explained by the existence of general as well as specific permeases. Such a case has been clearly demonstrated for the aromatic amino acids in *Salmonella* (Ames, G. F. 1964 Arch. Biochem. Biophys. 104: 1).

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Because of this abnormal excess of cytochrome c, this system is ideal to study the regulation and synthesis of this protein.

Extraction of cytochrome c with borax (Hardesty 1961 Ph. D. Thesis, California Institute of Technology) and subsequent purification with $\left(\text{NH}_4\right)\text{SO}_4$ and column chromatography on either CM Sephadex 25 or Amberlite CG-50 gives two chromatographic species of cytochrome c. Both iso-cytochromes c* have a sedimentation coefficient of 1.5, thus ruling out the possibility that one species is a polymer. Peptide maps of the two proteins are nearly identical, but preliminary results with thin layer electrophoresis on silica gel show one or more differences between the tryptic peptides of the two proteins.

Table 1 illustrates the chromatographic elution profiles of cytochrome c extracted from three different ages of poky. Under the inoculation conditions used, the cultures were at the following stages of growth: 22 hours = pre-log growth; 38 hours = log growth; and 123 hours = post-log growth. As shown, iso-2-cytochrome c is the predominant species in young poky cultures, but at 38 hours of growth both cytochromes c are present in nearly equal concentrations. The ratio of iso-1-cytochrome c to iso-2-cytochrome c increases until iso-1-cytochrome c is the predominant form in the 123-hour culture. Older cultures of wild type also contain only iso-1-cytochrome c.

While the poky cytochrome c undergoes this sequential change, the mutant approaches a more normal phenotype. But the striking fact is, the cytochrome c excess is due to iso-2-cytochrome c. The drop in the cytochrome c level then parallels the appearance of iso-1-cytochrome c.

To answer the question whether iso-2-cytochrome c is converted to iso-1-cytochrome c or whether iso-1-cytochrome c is synthesized de novo, poky has been pulse labeled with uniformly labeled $\text{C}^{14}$-lysine at different ages. Both cytochromes are labeled when present with approximately the same specific activity. Young poky has been pulse labeled also with $\text{C}^{14}$-lysine and then...
allowed to grow in cold medium. Sampling the culture at different times during subsequent growth indicated that the label stays in the cytochrome c during the change from iso-2-cytochrome c to iso-1-cytochrome c.

Figure 1. Chromatography of cytochrome c isolated from different ages of poky. Partially purified cytochrome c was applied to a 1 cm. x 50 cm. CM Sephadex 25 column equilibrated with 0.05 M Tris buffer, pH 7.6. The cytochrome c was eluted with a linear gradient prepared by placing 200 ml. of 0.05 M Tris buffer, pH 8.6, in the mixing chamber and 200 ml. of 1.0 M Tris buffer, pH 8.6, in the reservoir.

We conclude that there are two cytochromes c in poky and that they are sequentially produced. Our labeling data further indicates that iso-2-cytochrome c is converted to iso-1-cytochrome c. * * * Biology Division, California Institute of Technology, Pasadena, California.

* The term "iso-cytochromes" was originally used by P. Slominski, et al. See, for example, A. A. Sel, et al. 1965 Biochim. Biophys. Acta 95: 486.