A phenylalanine permease system in Neurospora crassa

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Abstract
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Perhaps the chief value of these results lies in the fact that with furfural one has a selective system. The germination by the use of furfural may be utilized as a selective technique when only certain genotypes are required from a sample of spores; for instance, in the selection of rare prototrophs from an interallelic cross. - - - Department of Biology, Queen's University, Kingston, Ontario, Canada.

DeBusk, B. G. and A. G. DeBusk. A phenylalanine permease system in Neurospora crassa. During recent years much work has been done on bacterial permeases. Cohen and Monod in 1957 (Bacteriol. Revs. 21:169) published their model describing a permease system in E. coli. Britten and McClure (1962 Bacteriol. Revs. 26:292) extended the information on E. coli permeases and further elaborated on the role of "amino acid pools" in the overall permease process. Many other workers have contributed to the knowledge of bacterial permeases and pools so that a comprehensive picture of the operation of these permeases is beginning to emerge.

Zalokar (1961 Biochim. Biophys. Acta 46:423), Lester and Hechter (1959 Proc. Natl. Acad. Sci. U. S. 45:1492) and Kinsky (1961 Biochem. Biophys. Res. Commun. 4:353) have published papers concerning transport in Neurospora crassa. However, since the volume of work published on Neurospora in no way parallels the amount of data available on bacteria, we felt further investigations might be helpful in understanding the role of permeases in Neurospora crassa. We have selected a phenylalanine permease system for extensive study.

Employing a basic incubation mixture containing conidia of strain ST74A, Cl4-phenylalanine in Vogel's salts minimal without sucrose or glucose, it has been found that the uptake of phenylalanine is temperature dependent and is destroyed by heat treatment. It is also pH dependent, having an optimal pH of 5.5. The Km was found to be 10-4 M. The permease shows stereospecificity. The system can concentrate phenylalanine to an internal concentration 700x greater than the external concentration.

Because of the high endogenous activity of the conidia, we have been unable to show a direct energy requirement. However, use of uncoupling agents, sodium azide and dinitrophenol, leaves little doubt that both the uptake process and the maintenance of the concentration gradient (pool) are energy-dependent.

These observations lead to the conclusion that the phenylalanine permease is an enzyme or a component of a system for which the rate-limiting step is enzymatic in nature. The "permease" enzyme(s) is linked to an energy-generating source.

If the uptake of phenylalanine is carried out in the presence of a carbon source such as glucose, the rate of uptake is markedly depressed. The amount of phenylalanine which is stored within the cell in the "free amino acid pool" is also much less while the amount of phenylalanine which is incorporated into protein is greatly enhanced.

It has been found that a number of other amino acids, tryptophan, tyrosine, methionine, leucine, fluorophenylalanine, norleucine and a-amino butyric acid, have a low inhibition index when competing with phenylalanine for the permease. Other amino acids show less competition and some show none. These may be grouped by the extent to which they inhibit into "families". These "families" show a high correlation with the ones determined by growth tests in which competition between phylalanine and other amino acids was studied using a phenylalanine mutant (E-5212) (Brockman, DeBusk and Wagner 1959 Arch. Biochem. Biophys. 84:455). These same amino acids compete with phenylalanine for occupation of the pool. The pool in which phenylalanine is stored appears to be expandable but has a definite limit in size.

We have been unable to obtain any evidence to indicate that the phenylalanine permease can be induced. An increase in activity can be produced by pre-incubation of the conidia alone. However, since this is not enhanced by the inclusion of phenylalanine, it is felt that this pre-incubation is affecting the energy-generating steps rather than increasing the activity of the permease.

We have examined the permease system of a number of N. crassa mutants which are resistant to p-fluorophenylalanine. Included in this group are three mutants obtained from D. Stadler which were isolated as 4-methyltryptophan-resistant mutants and are also p-fluorophenylalanine-resistant. We
found that these mutants differed greatly in the activity of the phenylalanine (and p-fluorophenylalanine) permease. They ranged in activity from as low as 20% to greater than 100% when compared to the control (ST74A). Crosses between these mutants have indicated that there are at least 2 and possibly 3 loci responsible for resistance to p-fluorophenylalanine. This may mean that there are two or three different phenylalanine permeases available to the cell.

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Dutta, S. K. and V. W. Woodward. The alteration of a complementation pattern among pyr-3 mutants of Neurospora. Based on the presence or absence of aspartate transcarbamylase (ATC) activity, pyr-3 mutants fall into two classes: those with and those lacking in vitro activity. Most of the ATC^- mutants are non-complementing whereas all of the ATC^+ types complement with two ATC^- mutants (KS-43 and pyr^-3d). R. H. Davis and V. W. Woodward have proposed that the pyr-3 locus specifies two active sites on one protein, one effecting the conversion of carbamylphosphate (CAP) and aspartic acid to ureidosuccinic acid and the other the synthesis of CAP, presumably from CO₂, NH₃ and ATP. It has been postulated that the non-complementing, ATC^- mutants possess either no ATC protein or ATC protein damaged at both sites; the ATC^+ mutants have ATC protein damaged only at the CAP-synthesizing site, and mutants KS-43 and pyr^-3d contain ATC protein damaged at the transcarbamylase site.

Evidence to this end was obtained (Woodward and Davis 1963 Heredity 18: 21) by changing one of the ATC^-, non-complementing mutants, by ultraviolet irradiation, to an ATC^+ complementing type. According to ATC activity, complementation, and suppressibility by a mutant known to suppress only ATC^+ types, the new mutation took on the qualities of the ATC^+ mutants. The evidence supported the idea that one of the damaged, enzymatic sites had been repaired by partial reverse mutation.

The present paper concerns the successful attempt to alter the same non-complementing, ATC^- mutant (KS-23) at the second active site. All mutants were marked with second morphological mutations (col^-4 and al^-2). ATC^-, col^-4 mutants were treated with an LD-50 dose of ultraviolet and over-plated onto minimal agar covered with conidia of ATC^+, al^-2 mutants. Colonies emerging on such plates were either reversions or heterocaryons. Heterocaryon formation was verified by the recovery of both homocaryons and the reconstruction of a heterocaryon between an ATC^+ mutant and one of the homocaryotic components.

Three ATC^-, non-complementing mutants (KS-23, KS-6 and KS-139) were irradiated and over-plated onto conidia of five ATC^+ mutants (KS-10, 16, 20, 48 and 125). Six heterocaryons from 615 colonies were recovered; four of the heterocaryons resulted from KS-23 + KS-125 and two were from KS-6 + KS-125. Homocaryons derived from these heterocaryons were shown to be of two types: one, the original ATC^+ mutant, and the other a mutant capable of forming heterocaryons with ATC^+ mutants. By the criterion of complementation, the second component resembles KS-43 and pyr^-3d, since it failed to complement the original KS-43, while the other homocaryon (KS-125) did.

Tests are under way to determine whether the alteration in complementation pattern results from mutation within the original mutation (primary site, partial revertant) or from mutation at another site (secondary site, partial revertant).

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