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Abstract

A phenylalanine permease system in *Neurospora crassa*

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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, C^{14}-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 α-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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