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Abstract

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The permease concept of metabolite entry into cells was first proposed by Rickenberg and co-workers in 1956 (Ann. Inst. Pasteur 9: 829). The concept of genetically controlled stereospecific transport processes, coupled with the amino acid growth inhibition data now in the literature, stimulated experiments in our laboratory on amino acid transport. The techniques used for the experiments reported here are similar to those previously reported for the characterization of the phenylalanine permease system (DeBusk and DeBusk 1965 Biochim. Biophyr. Acta 104: 139).

Arginine is actively transported into Neurospora crassa (74A) conidio by a constitutive, stereospecific permease system showing characteristic Michaelis-Menton kinetics and a Km of 2 x 10^{-6} M. The Process is temperature-dependent with on optimum at 35°C. A pH optimum occurs at 5.6. The amino acid is transported against a concentration gradient, resulting in an intracellular orginine concentration some 1450-fold greater than that of the external medium. The transport process is energydependent as shown by its complete inhibition by NaN_3 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 characteristically 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 inhibiton, while glutamic acid was a poor inhibitor. The reduction in arginine transport at various inhibitor-to-arginine ratios is summarized in Table 1.

Table 1. Arginine transport expressed as % of control,

	10:1	20:1	50:1	100:1
Lysine	28.0	15.2		5.2
Ornithine		29.6	18.3	12.0
Histidine		40.0	27.5	28.0
Phenylalanine	43.5	42.6		33.6
Tryptophan		32.0	32.0	27.5
Citrulline		55.4	41.6	36.0
Alanine	55.8	49.0		41.0
Isoleucine		54.0	44.5	37.2
Leucine		46.8	41.0	42.2
Methionine		44.5	40.0	39.2
Serine		68.0	55.0	47.0
Glutamic Acid		96.0	78.0	54.0
Glycine		75.5	60.3	53.0
Threonine		73.0	65.0	50.0
Proline		101.0	98.2	101.0

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) (rote independent of concentration). When lysine-C^{14} and arginine-C^{14} were simultaneously transported, the resulting rate was the overage 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-C^{14} and orginine-C^{14} were simultaneously transported. The initial rate of C^{14} transport was 80% of the sums 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 orginine 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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