Tryptophan Transport in *Neurospora crassa*

I. Specificity and Kinetics

W. R. WILEY AND W. H. MATCHETT

*Biology Department, Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, Washington*

Received for publication 18 August 1966

**ABSTRACT**

WILEY, W. R. (Battelle Memorial Institute, Richland, Wash.), AND W. H. MATCHETT. Tryptophan transport in *Neurospora crassa*. I. Specificity and kinetics. J. Bacteriol. 92:1698-1705. 1966.—The transport of tryptophan in *Neurospora crassa* is mediated by a distinct stereospecific system which is chemically specific for a family of neutral amino acids. The process shows typical saturation kinetics and a sharp decrease in the rate of tryptophan uptake at low temperatures. The $Q_{v}$ for the process is approximately 2 between 20 and 30 C. The apparent $K_{m}$ for uptake is $5 \times 10^{-4}$ M. Leucine and phenylalanine competitively inhibit the rate of tryptophan transport; the $K_{i}$ values are $1.1 \times 10^{-4}$ M and $4.0 \times 10^{-4}$ M, respectively. These data are interpreted as evidence that these amino acids are transported by the same transport site(s). Inhibition studies with amino acids and other compounds structurally related to leucine and phenylalanine suggest that an uncharged side chain and an α amino group, next to a carboxyl, represent three attachment points for the uptake site.

The cell membrane of *Neurospora crassa* is highly impermeable to neutral amino acids. This fact has led to the postulation of specialized mechanisms to account for the uptake of these amino acids. The precise mechanism of amino acid transport in *N. crassa*, however, remains obscure.

The rate of proline uptake by mycelial pads was investigated by Zalokar (12) and shown to be proportional to the external proline concentration. In addition, uptake was shown to occur against a large intracellular concentration gradient. These observations were interpreted as support for the contention that proline entry into mycelial pads is mediated by a specialized process.

Further support for the existence of specialized amino acid uptake systems was obtained from the study of phenylalanine transport in conidial suspensions of *N. crassa*. DeBusk and DeBusk (4) showed that conidia transport phenylalanine across the cell membrane by a process which is enzymatic in nature. Furthermore, mutant strains of *N. crassa* have been isolated which are defective in their ability to transport certain amino acids (7, 10, 11).

In the present report, we have directed our attention to a study of some of the biochemical properties of tryptophan transport in germinated conidia of *N. crassa*. Specifically, we were interested in obtaining evidence that a transport binding site exists which combines with tryptophan and controls its entry, and that this binding site shows both structural and stereochemical specificity for L-tryptophan, and in making some inferences concerning the minimal chemical structures required for reactivity with the transport site.

**MATERIALS AND METHODS**

*Organisms*. The strains of *N. crassa* used in these investigations were: wild type (WT) 74A; mutant strains td-201, auxotrophic for tryptophan, and 3357, auxotrophic for leucine and adenine.

*Growth and preparation of cells*. Stock cultures were grown and maintained on Vogel's mineral salts medium (WOW) supplemented with 2% sucrose and the appropriate growth factor(s).

Inocula for shake cultures were prepared by suspending conidia, from the surface of agar butts, in a small volume of WOW; the resulting suspensions were then aseptically filtered through sterile cheese cloth to remove mycelial fragments. Conidia (2 × 10⁷; per 500 ml of medium) prepared in this manner were immediately inoculated into WOW plus the appropriate supplement, and incubated, with vigorous aeration on a reciprocal shaker for 15 hr at 30 C. After 15 hr, the germinated conidia were harvested by filtration onto membrane filters (Millipore type HA, 0.65-µ pore size, Millipore Filter Corp., Bedford, Mass.) and washed twice, in fresh WOW, by repeated filtration. Cells

1698
prepared according to this standardized procedure were used in the transport experiments.

Transport experiments. The transport and accumulation of tryptophan were measured isotopically with L-tryptophan-3-C\(^{14}\) (New England Nuclear Corp., Boston, Mass.).

In a typical experiment, germinated conidia of the tryptophan auxotroph td-201, prepared as described previously, were resuspended (512 \( \mu \)g/ml of cells, dry weight) in WOW plus 10 \( \mu \)g/ml of L-tryptophan (unlabeled) and incubated at 30 C for 1 hr. After the 1-hr incubation period, the cells were again washed in cold (4 C) minimal WOW, resuspended in WOW plus 2\% sucrose, pH 5.8 (310 \( \mu \)g/ml of cells, dry weight), and kept at 4 C until they were used in the uptake experiments. This procedure provided cells with consistently reproducible rates of tryptophan transport. Cells starved of tryptophan transport tryptophan at a rate which is inversely proportional to the length of the starvation period.

For convenience of sampling, the uptake experiments were conducted in open beakers contained in a constant-temperature water bath. The use of an immersible magnetic stirring device insured vigorous aeration of the cells.

To initiate the uptake experiments, test amino acids contained in a small volume of water were rapidly added to the cell suspensions. Two 2-ml samples were removed from the reaction mixture at each sampling interval, usually 10 to 30 sec, with calibrated Cornwall pipettes. One sample was immediately filtered onto a membrane filter (Millipore type HA, 0.45 \( \mu \)), and the other was added directly to an equal volume of 10\% trichloroacetic acid. The radioactivity associated with the cells which were immediately filtered provided a measure of the total incorporation of labeled amino acid (1). Cells washed in cold (4 C) WOW medium were used to correct for extracellular radioactive contamination. Intracellular (pool) tryptophan was not removed in the washing process.

After 30 min, the samples treated with trichloroacetic acid were filtered and washed with 5\% trichloroacetic acid containing 500 \( \mu \)g/ml of unlabeled tryptophan. The radioactivity associated with these samples gave a measure of the incorporation of label into protein.

All radioactivity measurements were made in a Packard Tricarb liquid scintillation counter (50\% efficiency) by immersing the filters directly into the scintillating liquid [POPOP (1,4-bis-2-(5-phenyl-oxazolyl)-benzene), 0.1 g; and PPO (2,5-diphenyl-oxazole), 4 g in 1 liter of toluene; 10 ml per vial]. Samples were counted for 10 min, or for a time adequate to give at least 2,000 counts per min per sample.

Intracellular state of accumulated tryptophan. The chemical state of the radioactive material in cells incubated with L-tryptophan-3-C\(^{14}\) was determined by subjecting cell extracts to chromatographic analysis. After 5 min of incubation in 10\(^{-7}\) M L-tryptophan-3-C\(^{14}\) at 30 C, cells were immediately collected on membrane filters (Millipore Filter Corp.), washed twice with 20 ml of cold WOW (4 C), resuspended in hot water, and boiled for 10 min. The resulting cell extracts were filtered and chromatographed on Whatman no. 3 paper. The developing solvent was composed of methanol, butanol, benzene, and water (2:1:1:1, v/v). In all cases, the major radioactive component was chromatographically indistinguishable from authentic L-tryptophan.

Large tryptophan pools (15 to 100 \( \mu \)moles per g of cells, dry weight) were determined with tryptophanase (5).

**RESULTS**

Time course of tryptophan uptake. The time course of tryptophan transport in the tryptophan auxotrophic mutant, strain td-201, is shown in Fig. 1. Tryptophan uptake began immediately after the addition of tryptophan to the uptake medium. Accumulation of tryptophan continued at a linear rate until the supply in the medium approached depletion. Soluble pool tryptophan rose rapidly at first, remained constant for approximately 3 to 4 min, and finally diminished as pool tryptophan was incorporated into protein. The initial incorporation of exogenous tryptophan into trichloroacetic acid-precipitable material was preceded by a lag of 90 to 120 sec. Incorporation of label after the lag was linear and rapid. These results suggest that exogenously

![Fig. 1. Time course of L-tryptophan uptake and incorporation into protein by Neurospora crassa mutant strain td-201. The reaction mixture contained 310 \( \mu \)g/ml of germinated conidia (dry weight) suspended in WOW plus 2\% sucrose and 2.1 \( \times \) \( 10^{-5} \) M L-tryptophan-3-C\(^{14}\) (specific activity, 2.12 \( \mu \)c/\( \mu \)mole), final pH 5.8. Soluble pool tryptophan is the difference between the total radioactivity in the cells and trichloroacetic acid-insoluble radioactivity.](http://jb.asm.org/Downloaded_from)
supplied tryptophan enters the cell and accumulates in an intracellular metabolic pool, and that entry into the metabolic pool is a necessary step for its incorporation into protein.

The uptake of tryptophan is essentially unidirectional. The results of an experiment designed to test the exchange between intra- and extracellular tryptophan are shown in Fig. 2. In this experiment, cells of \textit{N. crassa} strain td-201 were preloaded with \(1.0 \times 10^{-4} \text{ M} \) L-tryptophan-3-C\(^{14}\) (specific activity, \(7.4 \times 10^{6} \text{ counts per min per } \mu\text{mole}\)) for 10 min, at 30 C. After 10 min, the cells were immediately cooled to 4 C, washed in cold minimal (WOW) medium, and resuspended in WOW plus 2\% sucrose. Cells preloaded in this way contained approximately 28 \(\mu\text{mole}\) of C\(^{14}\)-tryptophan per g of cells, dry weight (hot water-extractable tryptophan; specific activity, \(2.43 \times 10^{6} \text{ counts per min per } \mu\text{mole}\)). The efflux of C\(^{14}\)-tryptophan was measured in the presence and absence of \(5 \times 10^{-5} \text{ M}\) unlabeled L-tryptophan.

As shown in Fig. 2, the rate of C\(^{14}\)-tryptophan efflux in the presence of external, unlabeled tryptophan is quantitatively insignificant (line C). Actually, the rate of tryptophan efflux does not differ markedly from the normal leakage of tryptophan from cells (line B). The concentration of C\(^{14}\)-tryptophan incorporated into protein during the course of the incubation was of minor significance relative to the size of the soluble pool.

In sharp contrast to the extremely low rate of tryptophan efflux, there was a rapid influx of external tryptophan, under the same experimental conditions (line A, Fig. 2). The rate of tryptophan influx was measured by preloading cells in the manner described for the efflux experiment, except unlabeled tryptophan (\(10^{-4} \text{ M}\)) was used. After preloading the cells, tryptophan influx was measured by supplying \(5 \times 10^{-5} \text{ M}\) C\(^{14}\)-tryptophan to the uptake medium. It seems clear from these results that the efflux of tryptophan occurs at a rate which is entirely too low for exchange diffusion (6) to account for the rapid uptake of isotopically labeled tryptophan. In fact, the data suggest that the transport of tryptophan is unidirectional. Similar results were observed in the leucine auxotrophic mutant, strain 33757. Using an entirely different approach, Matchett and DeMoss (9) arrived at the same conclusion.

**Dependence of the uptake process on substrate concentration.** The rate of tryptophan uptake, in germinated conidia of \textit{N. crassa}, increases with increasing external tryptophan concentration. The initial velocity, however, does not increase indefinitely but tends to become saturated. The kinetics of uptake in mutant strain td-201 are shown in Fig. 5. There is a proportionality (open triangles) between the reciprocal of the initial rate of uptake and the reciprocal of the external tryptophan concentration. The adherence of the uptake process to Michaelis-Menton kinetics suggests that it is enzymatic in nature. The apparent \(K_m\) and \(V_{max}\) for the process are \(5.0 \times 10^{-4} \text{ M}\) and 33 \(\mu\text{mole}\) per min per g of cells (dry weight), respectively. The variation in the values obtained for \(K_m\) and \(V_{max}\) in three independent experiments, was approximately 16\%.

Conceivably, the saturable component could represent an obligatory intracellular binding site unrelated to the transport process. This possibility was partially eliminated by demonstrating that the initial rate of tryptophan uptake is maximal at an external tryptophan concentration, which is at least 10-fold less (assuming 90\% \(V_{max}\) is obtained at [S] corresponding to \(K_m \times 10\) ) than that necessary for saturation of the intracellular tryptophan pool (\(5 \times 10^{-5} \text{ M}\)). It appears from these results that, although tryptophan transport and

![Fig. 2. Rate of exchange between intra- and extracellular tryptophan. Germinated conidia (6.2 mg of cells (dry weight) per ml) of strain td-201 were preloaded for 10 min at 30 C, with \(1.0 \times 10^{-4} \text{ M} \) L-tryptophan-3-C\(^{14}\) (specific activity, \(7.4 \times 10^{6} \text{ counts per min per } \mu\text{mole}\)). Lines B and C show plots of the efflux of L-tryptophan-3-C\(^{14}\) as a function of time in the presence (line C) and absence (line B) of \(3 \times 10^{-4} \text{ M} \) unlabeled L-tryptophan contained in WOW plus 2\% sucrose medium. Line A shows the time course of tryptophan uptake by cells treated in identically the same manner as described above, except that they were preloaded with \(10^{-4} \text{ M}\) unlabeled tryptophan, and the rate of uptake was measured with \(5 \times 10^{-5} \text{ M} \) C\(^{14}\)-tryptophan (specific activity, \(2 \times 10^{6} \text{ counts per min per } \mu\text{mole}\)).

\[
\text{A} \quad \text{tryptophan uptake} \quad \text{D}
\]
\[
\text{B} \quad \text{tryptophan efflux} \quad \text{X}
\]
\[
\text{C} \quad \text{tryptophan efflux, \text{in presence of } 5 \times 10^{-5} \text{ M external tryptophan} \text{, or } \text{in absence of } 5 \times 10^{-5} \text{ M external tryptophan}.}
\]
intracellular pool maintenance in \textit{N. crassa} may be physiologically linked, they are mediated by two distinct processes. Additional evidence in support of this contention will be discussed later in the text.

\textit{Effect of temperature and pH on tryptophan transport.} The initial rate of tryptophan uptake in \textit{N. crassa} (strain td-201) increased progressively with increasing temperature from 0 to 40 C (Fig. 3). The $Q_90$ calculated between 20 and 30 C is approximately 2.0. There was a marked decrease in the rate of uptake at 46 C, and transport was completely eliminated at 56 C. The decrease in the uptake rate at low temperatures was greater than would be expected for thermal diffusion. The rates shown in Fig. 3 were obtained from time-course studies of the type shown in Fig. 1.

Even though the rate of transport at 0 C was practically nil, pools formed at 30 C were retained at 0 C. Figure 4 shows the stability of the tryptophan pool at 0 C. In this experiment, cells were preloaded with $4.8 \times 10^{-3}$ M tryptophan at 30 C, chilled to 0 C, washed free from exogenous tryptophan, and incubated at 0 and 30 C. The intracellular tryptophan pool was measured and plotted as a function of time. The decay of the tryptophan pool observed at 30 C was not a result of tryptophan leakage (Fig. 2), but resulted from (i) the incorporation of tryptophan into protein and (ii) the conversion of tryptophan to other intermediates of the tryptophan cycle (8).

The transport of tryptophan was also pH-dependent. The optimal pH range for uptake was between pH 5.8 and 6.2. The rate of uptake decreased at pH values below 5.0 and above 7.5.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Effect of temperature on the rate of tryptophan transport. The rates of uptake at each temperature were obtained from the same cell preparation. Mutant strain td-201 was used in the experiment. Time-course studies were conducted according to the procedures described previously (Fig. 1). The external L-tryptophan concentration was $1.0 \times 10^{-3}$ M (specific activity, 4.17 $\times 10^6$ counts per min per \textmu{}mole).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Retention of intracellular tryptophan pools at 0 C. Cells of \textit{Neurospora crassa} mutant strain td-201 [620 $\mu$g (dry weight)/ml] were preloaded (for 30 min) with $4.8 \times 10^{-3}$ M unlabeled tryptophan in minimal WOW medium. After 30 min of incubation at 30 C, the cells were chilled to 0 C and washed twice by repeated filtration in cold minimal WOW. The washed cells were then resuspended [620 $\mu$g (dry weight)/ml] in WOW plus 2% sucrose, and the decay of the hot water-extractable pool was measured and plotted as a function of time at 0 and 30 C. Tryptophan was measured with tryptophanase.}
\end{figure}

These observations provide further support for the hypothesis that tryptophan transport in \textit{N. crassa} is mediated by a catalytic site of limited availability. The fact that pools are maintained at 0 C, at a temperature at which uptake is nil, may be interpreted as additional evidence that the entry and maintenance processes are two distinct systems.

\textit{Specificity of the transport process.} One of the principal characteristics of enzyme-catalyzed reactions is the strict limitation of their action to a single substance or to a very small number of closely related substances. To strengthen the inference concerning the enzymatic nature of the tryptophan transport process in \textit{N. crassa}, the degree of specificity of the process was investigated. Table 1 shows the effect of several other amino acids on the rate of tryptophan transport. L-Phenylalanine, leucine, methionine, ethionine, cysteine, and tyrosine inhibited the rate of tryptophan uptake 80 to 95%; at a ratio of tryptophan to inhibitor equal to 1:50 to 1:40. Serine and histidine inhibited uptake by approximately 50%. The other amino acids were only slightly inhibitory (0 to 20%), if at all. The D-isomers of tryptophan and leucine illustrate the stereospecificity of the transport system. Steric specificity is absolute; no inhibition in the rate of L-tryptophan uptake by the D-isomers was observed. Actually, D-tryptophan was slightly stimulatory. Similar results have been observed for wild-type strain 74A (7). Presently there is no explanation for this phenomenon.

The nature of L-leucine inhibition was further
Table 1. Effect of other amino acids on tryptophan uptake

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>Percent Inhibition of Initial Rate of Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Leucine</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>5</td>
<td>82</td>
</tr>
<tr>
<td>L-Ethionine</td>
<td>5</td>
<td>82</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>5</td>
<td>77</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>5</td>
<td>72</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>4</td>
<td>47</td>
</tr>
<tr>
<td>L-Serine</td>
<td>4</td>
<td>53</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>L-Valine</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Glycine</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>D-Tryptophan</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>D-Leucine</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

* External tryptophan, 10^{-4} M; the inhibitor and tryptophan were added simultaneously to the reaction mixture. The rate of uptake was calculated from time-course studies of the type shown in Fig. 1.

investigated, and the results are shown in Fig. 5. The double reciprocal plot of tryptophan concentration versus initial velocity in the presence and absence of 5.0 \times 10^{-4} M L-leucine shows that leucine is a competitive inhibitor of transport. The apparent \( K_i \) for leucine inhibition is approximately 1.1 \times 10^{-4} M. Moreover, as shown by the Lineweaver-Burk plot in Fig. 6, phenylalanine is also a competitive inhibitor of tryptophan transport. The inhibition constant for L-phenylalanine is approximately 4.0 \times 10^{-4} M. Although there were some methodological limitations in the determination of the inhibition constant \( (K_i) \), the results were quite reproducible; replicate experiments were within 10 to 15% of the above values. These results immediately suggest that L-phenylalanine, L-tryptophan, and L-leucine are transported across the cell membrane by a common transport site. Additional evidence in support of this suggestion is the fact that tryptophan inhibits the rate of leucine transport. Moreover, DeBusk and DeBusk (4) have shown that phenylalanine uptake is markedly inhibited by L-tryptophan and leucine. These competitive relationships were observed in all strains of \( N. \ crassa \) tested (td-201, WT-74A, and 33757).

The results of an experiment designed to show that leucine competes with tryptophan for transport and not its utilization or retention are shown in Fig. 7. In this experiment, \( N. \ crassa \) mutant strain td-201 was preloaded with L-tryptophan and washed free from extracellular tryptophan; the disappearance of the intracellular pool was measured and plotted as a function of time at 30 C. As shown, leucine \( (4 \times 10^{-4} M) \) was without effect. Pool tryptophan was assayed by the tryptophanase method. The data show that the utilization of tryptophan was unaltered by the addition of external leucine. Increasing the external leucine concentration to \( 4 \times 10^{-4} M \) also failed to inhibit the rate of utilization of the pre-formed tryptophan pool. Additionally, no appreciable tryptophan efflux was observed. Thus, the inhibitory effect of leucine appears to be restricted to its effect on the transport system.

The results described above are consistent with the proposal that tryptophan transport in \( N. \ crassa \) is mediated by a distinct, stereospecific process which is enzymatic in nature. Further, the data suggest that the transport site is specific for a limited number of naturally occurring amino acids.

![Fig. 5. Competitive inhibition of tryptophan transport by leucine. The reciprocal of the initial velocity of tryptophan transport (v) is plotted against the reciprocal external tryptophan concentration (S). Leucine was added at a concentration of 5 \times 10^{-4} M. The organism used was mutant strain td-201. The experimental conditions were the same as those described for Fig. 1.](http://jb.asm.org/Downloaded from http://jb.asm.org/ on August 15, 2017 by guest)
modifications in the functional groups common to the tryptophan transport family for their ability to inhibit competitively tryptophan uptake. Initially, it was desirable to determine whether the indole portion of the tryptophan molecule contributed to the structural specificity of the site. To test this notion, the inhibitory effect of indole on the transport site was examined; the results are shown in Table 2. Indole (4 x 10^{-4} M) and 2 x 10^{-4} M L-tryptophan-C\(^{14}\) were added simultaneously to the uptake medium containing 310 \(\mu\)g/ml of cells, and the effect of indole on the rate of uptake was measured. Indole was not an inhibitor of uptake; increasing the concentration in the medium 10-fold also failed to inhibit uptake. Actually, the presence of indole in the uptake medium may be slightly stimulatory. The rate of tryptophan uptake in the absence of an inhibitor was 10 \(\mu\)moles per g of cells (dry weight) per min. It appears from these results that the specificity of the uptake site resides in the amino acid side chain.

### Table 2. Minimal structural requirements for reactivity with the transport site

| Competitor | Conc (X 10^{-4} M) | Initial rate of tryptophan uptake
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Reactivity of the indole moiety</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Effect of substitutions on the carbon (\beta) to the (\alpha) carboxyl of the amino acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Leucine</td>
<td>4</td>
<td>0.96</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>4</td>
<td>9.6</td>
</tr>
<tr>
<td>L-Valine</td>
<td>4</td>
<td>9.0</td>
</tr>
<tr>
<td>Requirement for an (\alpha) amino group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indolepyruvic acid</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>L-[(\alpha)] Amino butyric acid</td>
<td>4</td>
<td>1.96</td>
</tr>
<tr>
<td>L-[(\gamma)] Amino butyric acid</td>
<td>4</td>
<td>9.6</td>
</tr>
<tr>
<td>Requirement of an (\alpha) carboxyl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptamine-HCL</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Effect of an electrically charged side chain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

\(a\) External tryptophan, 2 x 10^{-5} M. The inhibitor and tryptophan were added simultaneously to the reaction mixture. Rates of uptake were calculated from time-course studies of the type described in Fig. 1, and are expressed as micromoles per gram of cells per minute.
To approach the question of the involvement of the β-CH₂ group in the process, amino acids which differed primarily in their configuration around the β carbon were tested for their ability to inhibit tryptophan uptake. As indicated in Table 2, leucine, which has a configuration around the β carbon analogous to that of tryptophan, was an extremely effective inhibitor of uptake. In contrast to leucine, isoleucine and valine were poor inhibitors of the rate of tryptophan uptake (Table 2). These results may be interpreted as evidence for the participation of the β carbon in the attachment of the amino acids to the transport site.

To examine the role of the amino group in the uptake process, indolepyruvic acid was tested for its ability to inhibit the rate of transport. The data in Table 2 show that indolepyruvic acid, like indole, is not only noninhibitory but may in fact be slightly stimulatory. The requirement of an amino group was further illustrated by the fact that (α) aminobutyric acid was a competitive inhibitor of the rate of tryptophan uptake (Table 2), whereas (γ) amino butyrate was completely noninhibitory. Thus, not only is there a necessity for an amino group, but the amino group must be present in the α position (α with respect to the carboxyl); the inhibitory effect of the amino group in the β position was not investigated.

The participation of the carboxyl group in the uptake process was examined by testing the effect of tryptamine (Table 2) on the transport of tryptophan. The rate of tryptophan uptake was unaffected by external tryptamine. We inferred from these results that an amino group next to a carboxyl and a CH₂ group in the position of the β carbon are the minimal structural requirements for reactivity with tryptophan transport site. There are, however, other factors which may contribute to the specificity of the site. For example, neither lysine nor glutamic acid was an inhibitor of tryptophan uptake (Table 2), yet they have the minimal structures necessary for reactivity with the site. In this same context, it should be pointed out that competition occurs between the dicarboxylic amino acids and between basic amino acids, but these do not compete with each other or with electrically neutral amino acids. Apparently, there is an additional requirement of an uncharged side chain for reactivity with the tryptophan transport site. A similar relationship between the neutral, diamino and dicarboxylic acids has been observed in Ehrlich ascites cells (2, 3).

**DISCUSSION**

We conclude from these studies that there exists in *N. crassa* a distinct stereospecific transport system for the unidirectional transport of tryptophan. The process appears to be enzymatic in nature and specific for a family of electrically neutral amino acids.

It appears evident from these investigations that the tryptophan entry mechanism and intracellular pool maintenance are two biochemically distinct processes. Evidence in support of this conclusion is the fact that two distinct conditions exist in *N. crassa* which permit maintenance of the pool when the rate of amino acid uptake is maximally depressed: (i) uptake is practically nil at 0 C while preformed (free tryptophan) pools are virtually undisturbed at 0 C, and (ii) externally supplied leucine inhibits the rate of tryptophan entry but has no effect on the maintenance of the pool. Additionally, the initial rate of tryptophan transport is maximal at much lower external tryptophan concentration than that required for the saturation of the intracellular pool.

The observation that exchange between external and internal tryptophan is absent in germinated conidia of *N. crassa* implies that exchange between the intracellular pool and the uptake binding site is also minimal. It is also conceivable that tryptophan exchange between the pool and the uptake site occurs, but exchange between the site and the environment is forbidden. Other explanations are equally probable. Future experiments are expected to provide a means for distinguishing between these possibilities.

Inhibition studies with compounds containing chemical modifications in the functional groups of amino acids, which comprise the tryptophan transport family, suggest that an α amino group next to a carboxyl and an uncharged side chain are minimal requirements for reactivity with the transport site. The implications are that the α amino, α carboxyl, and the uncharged side chain represent three points of attachment for the transport site. Studies, presently underway, are expected to provide additional information on the chemical nature of the transport site.

**ACKNOWLEDGMENTS**

The competent technical assistance of Mrs. L. S. Winn is gratefully acknowledged.

We express our gratitude to W. N. Ogata of the Fungal Genetics Center, Dartmouth College, for the gift of a culture of *N. crassa* mutant strain 33757.

This investigation was performed under contract no. AT(45-1)-1830 between the United States Atomic Energy Commission and the Battelle Memorial Institute.

**LITERATURE CITED**


2. Christiansen, H. N. 1960. Reactive sites and bio-


