SPECIFICITY OF TRANSINHIBITION OF AMINO ACID TRANSPORT IN NEUROSPORA

Martin L. Pall* and Katherine A. Kelly
Department of Biology, Reed College
Portland, Oregon 97202

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SUMMARY: Amino acid transport systems I and III in Neurospora are inhibited by amino acids in the intracellular pool (transinhibition). The transinhibition is system specific. The ability of an amino acid to transinhibit a transport system is highly correlated with its affinity for the system. The significance of the system specificity of transinhibition is discussed.

The active transport of amino acids has been found to be inhibited by amino acids in the intracellular pool in Streptomyces hydrogenans, Saccharomyces cervesiaie, and Neurospora crassa (1-5). Such inhibition may be important in the regulation of transport and has been called transinhibition. Ring, Gross and Heinz (3) have reported in studies on Streptomyces that the ability of an amino acid to transinhibit a transport system bears little relationship to the specificity of the system involved. However, Crabeel and Grenson (4) report that only histidine of the amino acids investigated appears to have the ability to transinhibit a specific histidine permease in yeast. The results of the histidine transport studies suggest a close relationship between ability to transinhibit a transport system and ability to be transported by it. Moreover, Pall (5) has found that in a methionine transport system in Neurospora, the ability to transinhibit the system is closely correlated with transport affinity for that system. Thus for the methionine transport system in Neurospora, transinhibition is system specific but for several systems in Streptomyces it apparently is not. The differences here raise the question as to what specificity of transinhibition is commonly found. The specificity, in turn,

*Author to whom reprint requests should be addressed.

may help to clarify both the function and the mechanism of transinhibition.

This investigation is a study of transinhibition in two amino acid transport systems in Neurospora crassa. The study will show that both the transport systems for L-neutral amino acids (system I) and for L-basic amino acids (system III) exhibit transinhibition which is highly system specific.

MATERIALS AND METHODS

Experiments were performed on wild type strain of Neurospora crassa ST74A or on the arginaseless strain aga (UM 906). The latter strain was obtained from the Fungal Genetics Stock Center. Growth of 2 day old mycelial pads and uptake measurements were performed as described previously (7). Preincubation involved adding amino acid to the growth medium and gently shaking the culture. In each experiment, all pads were shaken for the same period of time. Preincubation was terminated by washing the pads 3x with 20 ml of 1x Vogel's medium N salts containing 2% sucrose (9) and then placing them in 20 ml of the same medium.

RESULTS

Studies were confined to transport system I, the neutral amino acid transport system and transport system III, the basic amino acid transport system (6-8). Preincubation of mycelial pads with phenylalanine (table I) leads to the lowering of tryptophan transport (by system I). Other experiments (data not presented) show that preincubation with phenylalanine also leads to the lowering of transport of phenylalanine and leucine, other amino acids taken up by transport system I (6). Similarly, preincubation with lysine leads to a lowering of lysine transport (by system III). Arginine uptake is also lowered by lysine preincubation. As is also shown in table I, preincubation with amino acid lowers transport even in the presence of the inhibitor of protein synthesis, cycloheximide. Similar results were obtained using 50μg/ml of the inhibitor of protein synthesis blasticidin-S·HCl in place of the cycloheximide. Both cycloheximide and blasticidin-S are rapid and

Table 1

Effect of Preincubation on the Activity of Two Transport Systems

Additions to growth medium for preincubation	Transport system measured	Activity (% of level with no preincubation)	Efflux (%)
None	I	(100)	21.8
L-phenylalanine	I	49	18.1
cycloheximide	I	73	19.1
L-phenylalanine + cycloheximide	I	52	20.0
None	III	(100)	22.3
L-lysine	III	51	21.0
cycloheximide	III	77	21.9
L-lysine + cycloheximide	III	52	25.4

L-lysine, L-phenylalanine and/or cycloheximide were added to the growth media of 2 day mycelial pads of 74A at $2x10^{-4}$M, 10^{-3}M, and 10 µg/ml respectively. The pads were shaken for 15 minutes, washed, and the activity of system I or system III determined. System I activity was determined by measuring the 4 minute uptake of 10^{-4}M L-tryptophan in the presence of 10^{-3}M L-arginine (6). System III activity was determined by measuring the 2 minute uptake of $2x10^{-5}$M L-lysine in the presence of $5x10^{-2}$M glycine (7). When efflux was measured, pads were washed on a Buchner funnel after the uptake of labeled tryptophan or lysine and placed into fresh growth medium containing 10^{-3}M sodium azide and 10^{-3}M amino acid (tryptophan or lysine respectively). After shaking for 15 minutes, the pads were collected and washed on a Buchner funnel and extracted with 5% trichloroacetic acid (TCA). Aliquots of the medium and extract were counted to measure the efflux.

effective inhibitors of protein synthesis in Neurospora (10-11). One may conclude that the preincubation with amino acid produced an inhibition of uptake rather than only a repression of the synthesis of some protein involved in uptake.

The above discussion involves the tacit assumption that the preincubation produces an inhibition of transport rather than a stimulation of efflux of labeled amino acid. The assumption seems reasonable because Wiley and Matchett (12) and Roess and DeBusk (13) have reported the efflux of amino acids from Neurospora to be quite small. When the efflux of labeled tryptophan or lysine is measured, only about one fifth of the counts originally present in the cells is released in 15 minutes (table I, last column). Preincubation with cycloheximide or amino acid (phenylalanine or lysine) produces little or no effect on efflux. The results support the conclusion that there

is probably little efflux during the brief uptake of labeled amino acid and that preincubation with amino acid acts to inhibit transport rather than to stimulate efflux.

A kinetic analysis of the inhibition produced by preincubation with phenylalanine is shown in figure 1. The inhibition produced is primarily noncompetitive, indicating that the main effect of preincubation is to lower the velocity of transport rather than change the Michaelis constant (K_m). The noncompetitive nature of the inhibition also shows that the inhibition cannot be due to unlabeled amino acid remaining in the medium after washing since such remaining amino acid would inhibit competitively.

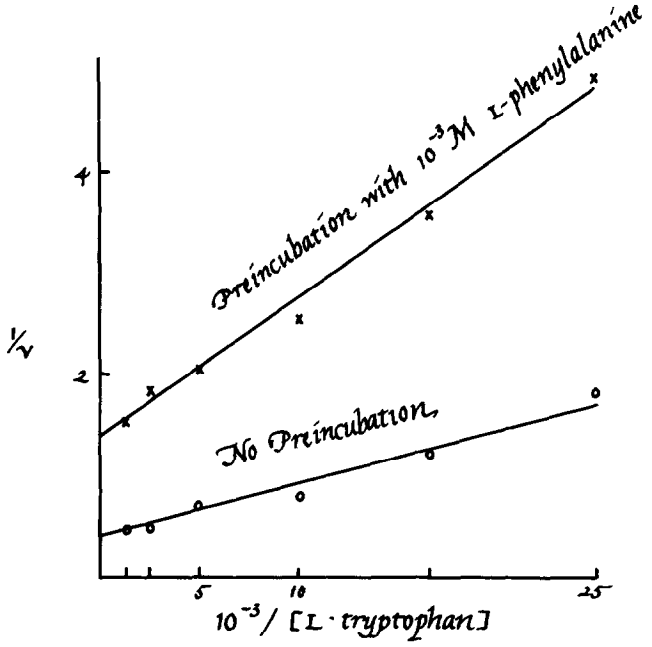

Figure 1. Two day old mycelial pads of wild type 74A were shaken for 15 minutes with or without 10^{-3}M L-phenylalanine. The pads were washed and the 4 minute uptake of labeled L-tryptophan in the presence of 10^{-3}M L-arginine was measured. v is expressed in nmoles of L-tryptophan taken up per mg dry weight of mycelium per minute.

When a variety of amino acids are preincubated to test their effectiveness in inhibiting transport, an interesting pattern emerges (table II). Only those neutral amino acids transported by transport system I effectively in-

Table 2

Preincubation with Various Amino Acids

Amino acid preincubated	Uptake (% of level with no preincubation)	
	System I	System III
None	(100)	(100)
L-phenylalanine	26	84
L-leucine	29	104
L-valine	56	82
L-alanine	62	76
glycine	78	80
L-arginine	103	23
L-lysine	101	31
L-aspartic acid	78	86
L-glutamic acid	71	79

Two day old mycelial pads of ST74A were preincubated with $5x10^{-4}$M amino acid for 30 minutes. The pads were washed, resuspended in fresh medium and the activity of each transport system was determined. The activity of system I was determined by measuring the uptake of 10^{-4}M L-tryptophan in the presence of 10^{-3}M L-arginine for 4 minutes (6). The activity of system III was determined by measuring the uptake of $2x10^{-5}$M L-arginine in the presence of $5x10^{-2}$M glycine for 2 minutes (7).

hibit system I. Furthermore, there is a good correlation between affinity for the system and the inhibition produced. L-Phenylalanine and L-leucine with the highest affinity for system I (6) produce the greatest inhibition of transport. Of the neutral amino acids tried, glycine has the lowest affinity for system I (Km $\sim 10^{-3}_{M}$) and produces the least inhibition of system I. Arginine and lysine with no affinity (6), produce no apparent inhibition of transport by system I. Aspartic and glutamic acids produce a small lowering of system I activity; it is possible that this lowering may be due to the stimulation of the synthesis of various neutral amino acids (14). It would appear that the ability to be transported by system I or the structural similarity to transported substrates determines the ability of a preincubated amino acid to inhibit transport. The apparent determination of inhibition by the structure of an amino acid supports the contention that it is the amino acid itself that is producing the inhibition and thus that inhibition by amino acid in the internal pool (transinhibition) is occurring here.

A study of transport system III (table II) produces a similar pattern.

Table 3

Concentrations of Amino Acids
Needed for 50% Transinhibition

Transport system	Amino acid preincubated	Concentration needed for 50% transinhibition	K_m(M)
I	L-phenyalanine	16.3	$.6x10^{-4}$
I	L-leucine	28.7	$1.2x10^{-4}$
I	L-valine	72.2	$4.7x10^{-4}$
III	L-arginine	19.3	$2.4x10^{-6}$
III	L-lysine	33.1	$4.8x10^{-6}$

Phenylalanine, leucine, valine, and lysine were used for preincubation because it was hoped that they would be metabolized less rapidly than most other amino acids. Preincubation with arginine and lysine was performed in the arginaseless strain to minimize metabolism of arginine. 10^{-3}M neutral or $2x10^{-4}$M basic ^{3}H labeled amino acids were used for preincubation, the amount of amino acid preloaded into the pool being estimated from the ^{3}H label measured later in the TCA extract. After preincubation, the pads were washed and shaken with 10^{-4}M (^{14}C) L-tryptophan in the presence of 10^{-3}M L-arginine or with $2x10^{-5}$M (^{14}C) L-arginine in the presence of $5x10^{-2}$M glycine to determine the activities of system I or system III respectively. Concentrations needed for 50% transinhibition were estimated as described in the text. Michaelis constants (K_m's) were determined as described previously (6,7). Transinhibition concentrations are expressed as nmoles per dry weight of mycelium.

Arginine and lysine, the two amino acids taken up by system III (7) are effective in transinhibiting it. The other amino acids tried show relatively little lowering of transport activity.

It seemed desirable to further quantify the relationship between the ability of an amino acid to transinhibit a transport system and its affinity for that system. In order to do this, mycelial pads were preincubated with amino acid for various periods of time, washed and the uptake of an amino acid by system I or system III measured. The amount of the preincubated amino acid extracted from the intracellular pool can then be compared with the amount of inhibition produced. The concentration of amino acid needed to produce 50% transinhibition could be estimated by interpolating between pads showing more than and less than 50% transinhibition. The detailed procedures used are described in table III. For the three amino acids studied with system I and the two amino acids studies with system III, there is an excellent correlation between affinity for the transport system and ability to transinhibit that system. The higher the affinity, the lower the concentration

needed for 50% transinhibition. Thus, the data in table II and the more quantitative data in table III support the proposition that transport systems I and III show system specific transinhibition.

DISCUSSION

The transport systems for neutral amino acids (system I) and for basic amino acids (system III) of Neurospora crassa (6-8) are inhibited by certain amino acids in the intracellular amino acid pool (transinhibition). There is a high correlation between the affinity of an amino acid for a transport system and its ability to transinhibit that system. This system specific transinhibition regulates the rate of active transport by the systems involved. The transinhibition found here for system I almost certainly accounts for the lowered transport produced by preincubation reported earlier by DeBusk and DeBusk (15) and by Wiley and Matchett (16).

The system specific regulation of transport by amino acids in the internal pool leads to the conclusion that a specific portion of each of these transport systems must have access to the inside of the cell. Presumably the regulation involves the binding of amino acids to some binding site of the transport system. The striking similarity between the specificity of transport and the specificity of transinhibition suggests that both may be determined by the same binding site. This could occur in a transport system with a single binding site if that site could change its access from the outside to the inside of the cell. A model for system specific transinhibition involving a single binding site is discussed elsewhere (5). Alternatively a transport system might have two or more identical binding sites one with access to the inside of the cell and one with access to the outside. If the specificity of transinhibition is determined by a binding site identical to the site determining transport specificity, then every transport substrate could regulate its own transport. Models of this phenomenon may shed light on the mechanism of active transport involved in systems showing system specific transinhibition.

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