BBA 75592

AMINO ACID TRANSPORT IN *NEUROSPORA CRASSA*

IV. PROPERTIES AND REGULATION OF A METHIONINE TRANSPORT SYSTEM

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(Received August 10th, 1970)

SUMMARY

A specific amino acid transport system in *Neurospora crassa* is described with affinity for methionine and several of its analogs. This methionine transport system has a Michaelis constant (K_m) for L-methionine of about 23 μM and has lower affinity for *S*-ethylcysteine, ethionine, *S*-methylcysteine and norleucine.

The methionine transport system has high activity under conditions of sulfur starvation and low activity under other conditions. It is regulated both at the level of protein synthesis and, more directly, at the level of activity. The synthesis of a protein needed for methionine transport system activity is regulated by the cys-3 locus, the synthesis being induced in the wild type by sulfur starvation. The methionine transport system is also inhibited by the presence of methionine and structurally similar amino acids in the internal amino acid pool (transinhibition). The ability of an amino acid to transinhibit is correlated with its affinity for the system. Amino acids with high affinity are effective transinhibitors, those with lower affinity are less effective transinhibitors. Most amino acids with no affinity for the methionine transport system show no transinhibition. A model is proposed for transinhibition in which the amino acid binds to the active site of a protein carrier on the inside of the membrane, inhibiting recycling of the carrier for further transport.

INTRODUCTION

Amino acids are the largest group of compounds commonly concentrated by active transport. Considerable progress has been made toward delineating the activity of the various amino acid transport systems in mammalian cells, bacteria and fungi. Relatively little is known, however, about the mechanisms by which such systems are regulated.

This report concerns the properties and regulation of a transport system for methionine in *Neurospora crassa*. Evidence is presented showing that this methionine transport system is regulated both at the level of synthesis of its protein and, more directly, at the level of its activity. Its synthesis is induced by sulfur starvation showing regulation similar to the sulfur pathway proteins. Its activity is inhibited by amino acids in the internal amino acid pool (transinhibition). The ability of an amino acid to

transinhibit the methionine transport system is closely correlated with its transport affinity for the methionine transport system. On the basis of this correlation, a model is suggested for transinhibition, in which the binding of amino acids to the active site of the transport system on the inner side of the plasma membrane leads to an inhibition of transport activity.

MATERIALS AND METHODS

Wild type strain ST74A was used in most experiments. Cys-5 (85518), cys-10 (39816) and me-2 (H 98) mutants requiring cysteine or methionine were obtained from Dr. N. H. Horowitz. Cys-3 (P22) was obtained from the Fungal Genetics Stock Center. L-[*Me*-^{14}C]methionine and L-[*Et*-^{14}C]ethionine were obtained from New England Nuclear.

Growth and uptake measurements were performed as described previously[1]. Where mycelial pads were washed, each pad was washed 3 times with 20 ml of deionized water and finally placed in 20 ml of sulfur free Vogel's medium N salts with 2 % sucrose. Amino acid analyses were run on 5 % trichloroacetic acid extracts using a Beckman 120 C amino acid analyzer. 0.2 M sodium citrate buffers, pH 3.2 and pH 4.25, were run on a UR-30 column at 51°.

RESULTS

General properties

Initial studies were confined to measurements of methionine uptake into 3-day sulfur-starved mycelial pads. The uptake was measured in the presence of a high concentration of glycine, the glycine being used to block methionine uptake[2] by transport Systems I and II*. The uptake of methionine into the trichloroacetic acid soluble pool of the cells proceeds linearly only for about 2 min (Fig. 1). At later times the net uptake appears to decrease. After 20 min, the methionine in the intracellular pool, as measured by amino acid analysis of the extract, is 11 times as concentrated as in the methionine in the external medium. In addition, a 5-min incubation with 10 mM sodium azide inhibits methionine uptake over 60 % and 5 min incubation with 1 mM dinitrophenol inhibits uptake about 75 %. The concentration of methionine from the

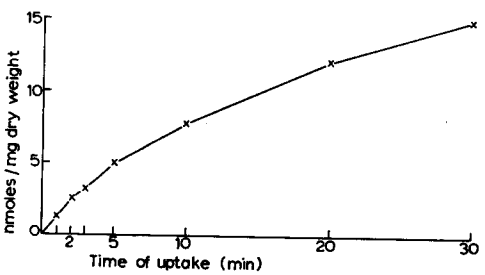

Fig. 1. Time-course of methionine uptake. The uptake of 0.1 mM L-methionine in the presence of 0.05 M glycine into sulfur-starved mycelial pads of ST74A was measured for various periods of time. Uptake is expressed as nmoles of L-methionine taken up per mg dry weight of mycelium.

* Glycine has a relatively poor affinity for System I (K_m about 1 mM) but since very high concentrations were used, it should still effectively block methionine uptake by System I.

medium and the sensitivity of the uptake to metabolic inhibitors supports the proposition that the methionine uptake involves active transport.

A large variety of amino acids in the medium show little or no influence on methionine uptake (Table I). Only amino acids structurally very similar to methionine, such as ethionine, homocysteine or norleucine, are effective inhibitors of methionine uptake. L-Methionine itself is the most effective inhibitor, indicating that the uptake involved is due to a specific methionine transport system. The relatively small inhibition exhibited by D-methionine indicates that this methionine transport system is stereospecific, preferring L- to D-stereoisomers.

TABLE I

UPTAKE OF L-METHIONINE IN THE PRESENCE OF VARIOUS AMINO ACIDS

All uptake performed in the presence of 0.1 M glycine. The uptake of 10 μM L-methionine was measured in the presence of 1 mM various unlabeled amino acids.

Unlabeled amino acid	*Methionine uptake (% of level with no unlabeled amino acid added)*
None	(100)
L-Arginine	94
L- 2,4-Diaminobutyric acid	109
L-Lysine	100
L-Aspartic acid	104
L-Glutamic acid	92
L-Alanine	100
L-Asparagine	92
L-Phenylalanine	92
L-Methionine	4.6
L-Ethionine	8.3
DL-Homocysteine	36
D-Methionine	51
DL-Norleucine	51
L-Cysteine	74
DL-Homoserine	112
DL-α-Amino-*N*-butyric acid	105

Kinetic studies of L-methionine uptake show that the transport involved follows Michaelis–Menten kinetics with a K_m for L-methionine of about 23 μM and a maximum velocity of transport of 1.7 nmoles/mg dry weight per min (Fig. 2). L-Ethionine is a competitive inhibitor of methionine uptake with a K_i of about 80 μM. Similarly the uptake of L-ethionine follows Michaelis–Menten kinetics with a K_m of about 100 μM; the uptake is competitively inhibited by L-methionine, the methionine showing a K_i of about 28 μM (Fig. 3).

The simple kinetics displayed in uptake studies of these two amino acids provides evidence that they are transported by a single transport system. In addition, there is excellent agreement between the K_m for methionine and its K_i when studied as an inhibitor of ethionine uptake. Similarly the K_m and K_i for ethionine are essentially equal. These equalities provide substantial support for the contention that

methionine and ethionine are taken up by the same transport system, designated here as the methionine transport system.

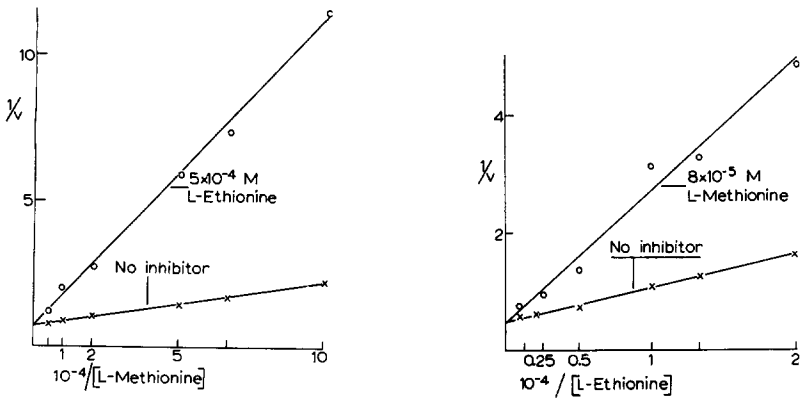

Fig. 2. L-Methionine uptake: inhibition by L-ethionine. The uptake of L-methionine for 2 min was measured in the presence or absence of 0.5 mM L-ethionine. All uptake was performed in the presence of 0.05 M glycine. v is expressed as nmoles taken up per min per mg dry weight of mycelium.

Fig. 3. L-Ethionine uptake: inhibition by L-methionine. The uptake of L-ethionine for 4 min was measured in the presence or absence of 80 μM L-methionine. All uptake was performed in the presence of 0.1 M glycine. v is expressed as nmoles taken up per min per mg dry weight of mycelium.

Further studies (data not presented) show that *S*-ethyl-L-cysteine, *S*-methyl-L-cysteine, and L-norleucine are competitive inhibitors of L-methionine uptake and thus appear to have an affinity for the methionine transport system. Kinetic studies have not been performed on other inhibitors of methionine uptake such as L-homocysteine or D-methionine but presumably these are competitive inhibitors as well. Methionine has the highest affinity for the transport system. The other amino acids, all structurally similar to methionine, have substantial affinity for the methionine transport system. The affinity constants (K_m or K_i) for five amino acids are shown in Table II.

TABLE II

AFFINITY CONSTANTS FOR METHIONINE TRANSPORT SYSTEM (K_m or K_i)

Each value listed where no standard deviation is given is the mean of three separate determinations. Each determination differed by no more than 30 % from the mean.

Amino acid	*Affinity constant* (μM)
L-Methionine	23 ± 4*
S-Ethyl-L-cysteine	49
L-Ethionine	92 ± 10*
S-Methyl-L-cysteine	260
L-Norleucine	300

* Standard deviation.

Uptake under different conditions

The above studies were performed using sulfur-starved mycelial pads. Carbon- and nitrogen-starved pads have very little methionine transport system activity, transporting methionine (in the presence of glycine) at about one fiftieth of the rate of sulfur-starved pads (Table III). It would appear that the methionine transport system is regulated in such a way as to have its highest activity under conditions of sulfur deprivation. The regulation is further demonstrated in Fig. 4. Growing pads that were washed and placed into sulfur free medium show a rapid increase in methionine transport system activity. This increase is blocked by the inhibitor of protein synthesis, cycloheximide. The induction of methionine transport activity by sulfur starvation is also inhibited by the inhibitor of protein synthesis, blasticidin S (Table IV). In addition, the amino acid analog, canavanine, effectively inhibits the increase in methionine transport system activity although its normal counterpart, arginine, has little effect. DL-*p*-Fluorophenylalanine is a more effective inhibitor of the increase in methionine transport system activity than is DL-phenylalanine. The effect of DL-phenylalanine is probably due to the toxic D-phenylalanine[3] since L-phenylalanine alone has little effect. It can be seen, then, that inhibitors of protein synthesis and amino acid analogs inhibit the induction of methionine transport system activity

TABLE III

ACTIVITY OF METHIONINE TRANSPORT SYSTEMS UNDER VARIOUS PHYSIOLOGICAL CONDITIONS

The uptake of 10 μM L-methionine was measured in the presence of 0.05 M glycine. v is expressed as nmoles of methionine taken up per mg dry weight per min.

Physiological state of culture	v
Sulfur-starved pads	0.435
Carbon-starved pads	0.0073
Nitrogen-starved pads	0.0105
2-day-growing pads	0.041

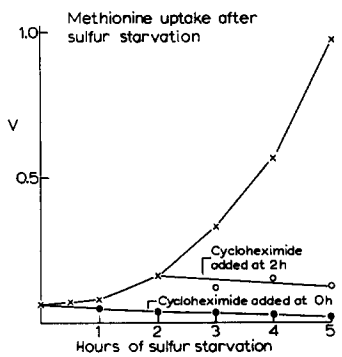

Fig. 4. Induction of methionine transport system. 2-day-growing mycelial pads of ST74A were washed and placed into sulfur-free medium containing 2 % sucrose. Cycloheximide at 10 μg/ml of medium was added as indicated. The uptake of 10 μM L-methionine in the presence of 0.05 M glycine was measured at various times after suspension in sulfur-free medium.

TABLE IV

UPTAKE OF $1 \cdot 10^{-5}$ M L-METHIONINE INTO TREATED GROWING PADS OF 74A

Growing mycelial pads of ST74A were washed and suspended in 20 ml of sulfur-free medium with 2% sucrose for 4 h. Where noted, various compounds were added to the sulfur-free medium immediately after the pads were suspended in it. The uptake of 10 μM L-methionine in the presence of 0.05 M glycine was measured after the 4 h of sulfur starvation.

Treatment	*Methionine transport*	
	nmoles/mg dry wt. per min	*% of level with sulfur starvation*
None	0.056	7.8
Sulfur starvation	0.745	(100)
Sulfur starvation + 10 μg/ml cycloheximide	0.037	5.1
Sulfur starvation + 20 μg/ml Blastidicin S	0.039	5.5
Sulfur starvation + 500 μg/ml DL-*p*-fluorophenylalanine	0.126	16.9
Sulfur starvation + 500 μg/ml DL-phenylalanine	0.253	34.0
Sulfur starvation + 100 μg/ml L-canavanine	0.055	7.7
Sulfur starvation + 100 μg/ml L-arginine	0.833	113

TABLE V

UPTAKE OF $1 \cdot 10^{-5}$ M L-METHIONINE INTO DIFFERENT STRAINS

3-day-old mycelial pads were grown in sulfur-free Vogel's medium N, 2% sucrose, supplemented with 25 μg/ml L-methionine. The uptake of 10 μM L-methionine was measured in the presence of 0.05 M glycine and is expressed as nmoles of methionine taken up per mg dry weight per min.

Strain	*Metionine uptake*
cys-3	0.0005
cys-5	0.385
cys-10	0.429
me-2	0.496
Wild type (74A)	0.427

produced by sulfur starvation. This supports the view that the induction involves the synthesis of a protein needed for methionine transport system activity*.

Further information on the regulation of this methionine transport protein can be obtained from studies of the cys-3 mutant. The cys-3 locus has been studied in detail by METZENBERG AND PARSON[4] and MARZLUF AND METZENBERG[5] and has been concluded to be a positive control locus regulating the synthesis of several sulfur pathway proteins. Each of these proteins is normally induced by sulfur starvation and is missing in cys-3 mutants. As is shown in Table V, the methionine transport system has normal activity in a number of cysteine and methionine mutants but is completely devoid of activity in the cys-3 mutants. Thus, the synthesis of methionine transport protein is regulated by the cys-3 locus. By analogy with the other proteins regulated by that locus, it can be concluded that methionine transport protein is induced by sulfur starvation. Both these studies and the studies with inhibitors of protein synthesis and amino acid analogs support the conclusion that the synthesis of methionine transport protein is regulated by the level of sulfur nutrition.

* It is possible, of course, that the synthesis of several proteins needed for methionine transport is involved.

Transinhibition

While the above results show that the methionine transport system is regulated at the level of protein synthesis, they do not, of course, eliminate the possibility of more direct regulation of its activity. As is shown in Fig. 1, the net uptake of methionine decreases with time of transport. One possible property which would produce such a decrease would be if methionine transported into the organism were to inhibit further methionine transport. Such inhibition by internal amino acids has been reported by RING AND HEINZ[6] in Streptomyces and has been called transinhibition. In order to search for possible transinhibition of the methionine transport system, mycelial pads were preincubated with unlabeled methionine, washed and then incubated with labeled methionine *plus* glycine*. It was found that preincubation with 0.1 mM L-methionine for 2 min inhibited the methionine transport system activity by about 40 %. The rapidity of this effect suggested that the inhibition occurred directly on the activity of the system rather than acting through the regulation of the synthesis of methionine transport protein. A similar conclusion can be derived from the results in Table VI. Significant transinhibition occurs even in the presence of cycloheximide. Since cycloheximide blocks essentially all protein synthesis in Neurospora, inhibition in the presence of cycloheximide cannot involve the regulation of protein synthesis. It can be concluded that at least part of the inhibition produced by methionine is due to a more direct effect on activity.

TABLE VI

PREINCUBATION WITH METHIONINE AND CYCLOHEXIMIDE: EFFECT ON METHIONINE UPTAKE

Preincubation was performed for 15 min with 1 mM L-methionine and 10 μg/ml of cycloheximide. After washing the mycelial pads, the uptake of 10 μM L-methionine in the presence of 0.05 M glycine was measured. Unpublished studies show that a 1-min incubation with cycloheximide (10 μg/ml) inhibits protein synthesis about 98 %.

Compound used for preincubation	*Uptake (% of level with no preincubation)*	
	Expt. 1	*Expt. 2*
None	(100)	(100)
L-Methionine	40	32
Cycloheximide	75	61
Cycloheximide + methionine	55	40

A kinetic analysis of the effect of preloading with methionine shows that the inhibition is approximately noncompetitive (Fig. 5). The small deviation from strictly noncompetitive inhibition was not generally found in other experiments. The noncompetitive nature of the inhibition indicates that the primary effect is due to a decrease in the maximum velocity of transport rather than due to a change in the K_m.

When a variety of amino acids are preloaded into the cell to test them as potential transinhibitors of the methionine transport system, an interesting pattern is seen (Table VII). A variety of neutral and acidic animo acids produce a small stimulation

* Preincubations were performed without glycine so that uptake by Systems I and II[2] as well as by the methionine transport system would occur.

of transport. The two basic amino acids tried produce a small inhibition of transport. The only amino acids which produce a substantial transinhibition are those amino acids transported by the methionine transport system. In addition, there appears to

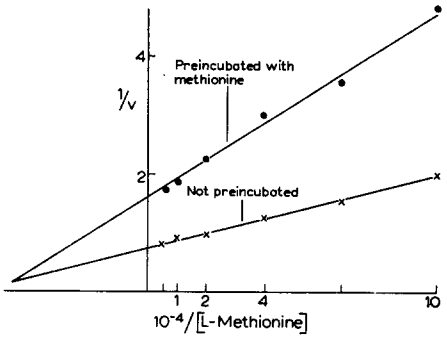

Fig. 5. Kinetic analysis of transinhibition. Sulfur-starved mycelial pads of ST74A were preincubated with 1 mM L-methionine for 15 min. Control pads were treated identically except that no methionine was used. The mycelial pads were washed, placed in 20 ml of sulfur-free medium containing 2 % sucrose. The uptake of L-methionine in the presence of 0.05 M glycine was measured. v is expressed as nomles taken up per min per mg dry weight of mycelium.

TABLE VII

TRANS EFFECTS OF AMINO ACIDS ON METHIONINE TRANSPORT SYSTEM

$1 \cdot 10^{-3}$ M various amino acids were preincubated with pads for 1 h. The pads were then washed, suspended in fresh sulfur-free medium; the uptake of 10 μM L-methionine was measured in the presence of 0.05 M glycine.

Amino acid used for preincubation	*Methionine uptake (% of level with no preincubation)*
None	(100)
L-Alanine	139
L-Asparagine	103
Glycine	119
L-Phenylalanine	123
L-Methionine	22
L-Ethionine	26
DL-Norleucine	31
L-Cysteine	71
L-Aspartic acid	122
L-Glutamic acid	137
L-Arginine	83
L-Lysine	60

be a correlation between the affinity of an amino acid for the methionine transport system and its ability to transinhibit. Methionine, with the highest affinity for the system is the best transinhibitor; ethionine with a lower affinity than methionine is a somewhat less effective transinhibitor; norleucine with still less affinity is still less effective as a transinhibitor; and cysteine with only very low affinity is a poor transinhibitor. This correlation between affinity for the methionine transport system and

ability to produce transinhibition suggests that it is the amino acids themselves which produce the transinhibition rather than some metabolite derived from them. Furthermore, their structural similarity to methionine or, more specifically, their ability to be transported by the methionine transport system seems to determine their ability to transinhibit.

Further studies were undertaken to quantify the relationship between affinity for the methionine transport system and ability to transinhibit. Mycelial pads were preincubated with an amino acid with affinity for the methionine transport system for various periods of time. The pads were washed and the uptake of methionine in the presence of glycine was measured. In most cases it was found that the longer preincubation proceeded, the greater the transinhibition produced. When mycelial pads, preincubated for some period of time, were found to have about 50 % transinhibition, their amino acid pools were measured by amino acid analysis to determine the concentration of preincubated amino acid needed to transinhibit 50 %. This concentration is shown for four amino acids in Table VIII. Methionine which has the highest affinity for the system is the best transinhibitor, having the lowest concentration for 50 % transinhibition. Ethionine with lower affinity requires a higher internal concentration for 50 % transinhibition. *S*-Methylcysteine with still lower affinity requires a still higher level. Only in the case of norleucine is there some deviation from this pattern,

TABLE VIII

TRANSINHIBITION BY FOUR AMINO ACIDS

Amino acids in 5 % trichloroacetic acid extracts were measured by amino acid analysis. Concentrations are expressed as nmoles per mg dry weight of mycelium

Amino acid	*Concentration for 50 % transinhibition*
L-Methionine	10.7
L-Ethionine	25.5
S-Methyl-L-cysteine	52.5
L-Norleucine	44.5

norleucine having slightly lower affinity for the methionine transport system than does *S*-methylcysteine (Table II) but transinhibiting 50 % at somewhat lower levels than *S*-methylcysteine. *S*-Ethylcysteine was also tested as a transinhibitor but since it does not separate from some other amino acids present in the free amino acid pool, accurate determination of its internal concentration at 50 % transinhibition was precluded. Nevertheless, its concentration for 50 % transinhibition did appear to be intermediate between concentration for methionine and the concentration for ethionine. Because its affinity for the system is intermediate between those of methionine and ethionine (Table II), this result also shows a correlation between ability to transinhibit and affinity for the methionine transport system. The studies with these five amino acids show an excellent correlation between affinity for the system and ability to transinhibit. The significance of this correlation is considered in the discussion of transinhibition below.

Role of transinhibition under different conditions

Because methionine is the only amino acid transported by the methionine transport system which should normally be present in substantial amounts, the measurement of the methionine in the internal amino acid pool should allow one to estimate the extent of transinhibition. Table IX shows the level of methionine present in the pool of several cultures. As expected, very little methionine is present in sulfur-starved cultures. However, growing cultures and carbon- or nitrogen-starved cultures have significant levels of methionine. Although some transinhibition would be expected to occur in these last three cultures, it should be noted that the internal concentrations of methionine are lower than that needed to produce 50 % transinhibition. Since the sulfur-starved cultures have from 10 to over 50 times higher activity than the other cultures (Table III), most of the difference in activity between the sulfur-starved cultures and the other cultures must be due to some regulation other than transinhibition. Clearly this other regulation is the regulation at the level of protein synthesis studied above.

TABLE IX

METHIONINE POOL IN VARIOUS CULTURES

Mycelial pads were extracted in 5 % trichloroacetic acid and the extracts examined by amino acid analysis. Pool sizes are expressed as nmoles per mg dry weight of mycelium.

Culture condition	*Methionine pool*
2-Day pads	3.25
Carbon-starved pads	9.5
Nitrogen-starved pads	3.93
Sulfur-starved pads	<0.5

Methionine concentration 10.7 for 50 % transinhibition

DISCUSSION AND CONCLUSIONS

General properties

Methionine transport in Neurospora is performed, in part, by a specific methionine transport system with affinity for methionine and several structurally similar amino acids. This system has a Michaelis constant (K_m) for L-methionine of about 23 μM and a maximum velocity of transport (v_{max}) of about 1.7 nmoles/mg dry weight per min in sulfur-starved mycelial pads at 25°. The methionine transport system varies in its activity, showing the highest activity in sulfur-starved mycelial pads and lower activity in growing and nitrogen- or carbon-starved mycelial pads. Most of the regulation of activity occurs through the regulation of the synthesis of a protein or proteins involved in methionine transport. The synthesis of this methionine transport protein is induced by sulfur starvation and its synthesis is controlled by the cys-3 locus, a locus which regulates the synthesis of several proteins involved in sulfur metabolism[4,5]. In addition to regulation at the level of protein synthesis, the methionine transport system is inhibited by the presence of methionine and other amino acids with affinity for that system in the free amino acid pool. Such inhibition by amino acids in the internal pool has been called transinhibition and is discussed in detail below. Lastly, a small effect of amino acids unrelated to methionine was found, most neutral and acidic

amino acids showing a stimulatory effect when loaded into the organism and the basic amino acids showing an inhibitory effect.

The methionine transport system differs from the other amino acid transport systems previously described in Neurospora by having relatively narrow specificity. Transport Systems I, II, III and IV each transport a wide variety of amino acids[1,2,7]. This difference in breadth of substrate specificity may be related to the functions served by the different systems. Apparently, the other systems mainly provide amino acids for protein synthesis or provide nitrogen or carbon under conditions of deprivation. These functions can be efficiently served by systems with broad specificity. The methionine transport system is regulated in such a way as to provide sulfur under conditions of sulfur deprivation. Since only a small number of sulfur sources may be available in the environment, it may be most efficient to have specific systems taking up each available source of sulfur. Methionine uptake by Systems I and II might be inhibited by a number of common amino acids, this inhibition will not occur with the methionine transport system.

The methionine transport system of Neurospora is very similar to a specific methionine transport system previously described in Penicillium by BENKO *et al.*[8]. The two systems are similar in their K_m for L-methionine and relative affinity for various amino acids. Both systems have much higher activity under conditions of sulfur starvation than under other conditions. BENKO *et al.* suggest that the methionine transport system in Penicillium is regulated by its being inhibited by a sulfur pathway intermediate before methionine. It seems more likely that the system in Penicillium, like that in Neurospora, may be regulated both by transinhibition by methionine and by regulation of the synthesis of methionine transport protein*. In yeast a specific methionine transport system has been characterized by GITS AND GRENSON[9] which is similar to those of Neurospora and Penicillium in substrate specificity but is active in cells grown in sulfur rich medium.

Transinhibition

Methionine and several other amino acids inhibit the methionine transport system when present in the free amino acid pools. Such inhibition by internal amino acids has been called transinhibition[6]. The transinhibition in the methionine transport system is system specific. Those amino acids transported by the methionine transport system give substantial transinhibition but those not transported by it do not. Furthermore, affinity for the system is correlated with ability to transinhibit. In most cases the greater the affinity of an amino acid to the system, the greater its ability to transinhibit.

One possible mechanism by which such transinhibition could occur would be if a protein involved in transport had an allosteric binding site for amino acids. Binding of amino acids could inhibit the activity of the protein involved and thus inhibit transport. By this mechanism, the transport system would have two binding sites, one binding the amino acid prior to transport into the cell and another binding transinhibiting amino acids.

* It would be interesting to test nonsulfur containing amino acids such as norleucine which apparently have affinity for the methionine transport system in Penicillium. to determine if they are capable of transinhibition in Penicillium. Such transinhibition would be predicted from the model of regulation proposed here but not from the model of regulation proposed by BENKO *et al.*[8].

Alternatively, the great similarity between transport specificity and transinhibition specificity may indicate that both are determined by a single active site. In this case, transinhibition could be envisioned to be caused by the binding of amino acid to the active site of the transport system when that site is oriented towards the inside of the cell. Such binding could inhibit recycling of the carrier, preventing reorientation of the active site towards the outside of the cell. A model of such a single active site mechanism is proposed in Fig. 6. The model involves a carrier protein designated C and an amino acid designated A. When the carrier is oriented towards the outside of the cell, it can reversibly associate amino acid *via* Reactions 1 and 2. The carrier–amino acid complex can change orientation from the outside to the inside of the cell by Reaction 3. The amino acid can then reversibly dissociate from the carrier by Reactions 5 and 6. Finally, the carrier can be recycled to the other membrane by a process involving ATP or other energy rich compound (Reaction 7). The critical assumption of the model is that for transinhibition to occur, Reaction 4 is slow compared with Reaction 3. This is equivalent to the assumption that the carrier–amino acid complex is in a lower energy state when oriented to the inside of the cell than when oriented to the outside. In transinhibition, amino acid with affinity for the active site accumulates inside the cell. The equilibrium between free carrier and carrier–amino acid complex is shifted by high concentrations internal amino acid toward the carrier–amino acid complex. The shift in equilibrium lowers the concentration of the free carrier, thus decreasing Reaction 7 and inhibiting the energy dependent cycling of the carrier. Because Reaction 4 is assumed to be slow, the carrier–amino acid complex cannot efficiently reorient itself toward the outer membrane, so recycling *via* Reaction 4 is low. It can be seen, then, that accumulation of amino acid with affinity for the active site inhibits recycling of the carrier and thus leads to transinhibition.

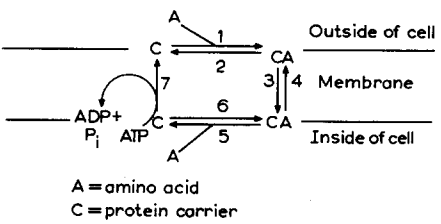

Fig. 6. A model for system specific transinhibition.

Under ideal conditions, the equilibrium constant for Reactions 5 and 6 might be expected to be equal to the equilibrium constant for Reactions 1 and 2 which might equal the K_m. If such ideal conditions were to hold, the concentration of an amino acid needed for 50 % transinhibition would be proportional to its K_m for transport. The data presented do not support this proportionality. There are a number of possible reasons why the behavior might be nonideal. For example, the equilibrium constant for Reactions 5 and 6 may deviate from the constant for Reactions 1 and 2 because of differences in pH, ionic strength or properties of the carrier on the two sides of the membrane. Also, different amino acids may bind differentially to internal components of the cell, causing their apparent internal concentrations to deviate from their intracellular thermodynamic activity. Consequently, it can be seen that the data for the

methionine transport system is consistent with the model presented here, even if it is not consistent with an ideal version of it.

The model presented here is not the only model which can be proposed in which the binding of intracellular amino acid to the active site of the transport system leads to transinhibition. It does have certain properties which make it particularly attractive. The assumption that Reaction 4 is slow leads not only to transinhibition but also leads to the conclusion that exchange diffusion must be slow. Unpublished results in this laboratory as well as the results of WILEY AND MATCHETT[10] show that there is little or no exchange diffusion of amino acids in Neurospora. Yeast appears to behave similarly to Neurospora not only with respect to transinhibition but also with respect to exchange diffusion[11]. If the remainder of the model is valid for yeast and Neurospora, the exchange diffusion results lead one to the conclusion that Reaction 4 must be very slow.

If the rate of Reaction 4 varies from one organism to another or from one transport system to another, a variety of transport properties can be accommodated by it. If the rate of Reaction 4 is high then one should find no transinhibition and should find considerable exchange diffusion. These findings have been obtained in Ehrlich cells by HEINZ AND WALSH[12]. The model they propose to explain their results is essentially identical to the model proposed here except that they assume the rate of reaction 4 to be high. If the rate of Reaction 4 is slow but significant, then one should find both transinhibition and exchange diffusion, the exchange diffusion being most easily seen under conditions where energy metabolism has been inhibited. Both exchange diffusion and transinhibition appear to be present in studies of α-aminoisobutyric acid uptake in Streptomyces[6,13]. In these ways, the variation of a parameter of a transport model may lead to the varying transport properties of different organisms.

The most important available evidence relating to the above discussion is that of system specificity of transinhibition*. Although other transport systems have not been studied in as much detail as the methionine transport system, substantial information is available on their transinhibition properties. The studies of WILEY AND MATCHETT[10] on transport System I in Neurospora, those by DYE[14] on System II, and unpublished results on System III, suggest that all these systems show system specific transinhibition. Similarly, the studies of CRABEEL AND GRENSON[11] in yeast demonstrate that the histidine permease shows system specific transinhibition. Investigations on purine and pyrimidine transport are somewhat more difficult to interpret than are the amino acid studies. However, the studies of CUMMINS AND MITCHISON[15] on adenine transport in yeast as well as those of GRENSON[16] on uracil and uridine transport in yeast suggest that the uptake of each of these compounds is controlled by system specific transinhibition.

Recently RING *et al.*[17] have reported on studies of transinhibition in Streptomyces in which they conclude that transinhibition is not system specific. From an examination of their results it appears that their studies on α-aminoisobutyric acid uptake may be consistent with the system involved being regulated by system specific transinhibition. Their results with glutamate and proline uptake appear not to be consistent with system specificity. Their findings raise the possibility of two different mechanisms for transinhibition: One showing system specificity, possibly acting by

* System specificity is used here to denote a correlation between affinity for a transport system and ability to transinhibit that system.

the model proposed above and one deviating from system specificity. The finding of inhibition by a compound which is not the substrate of a transport system should not be surprising. Such inhibition was reported by DREYFUSS AND PARDEE[18] to be involved in the regulation of sulfate transport in Salmonella. The question raised here is whether the mechanism of system specific transinhibition is different from the nonsystem specific phenomenon and whether the system specific transinhibition may occur through binding to the active site of the transport system along the inner surface of the membrane. The proposed mechanism for system specific transinhibition would clearly differ in important ways from enzyme regulatory mechanisms and might constitute a form of regulation unique to active transport.

ACKNOWLEDGEMENTS

I am indebted to Mr. Sam Martin, Miss Izzat Gulamani and Mrs. Katherine A. Kelly for their excellent assistance. I also wish to thank Dr. Richard Jones at the University of Oregon School of Medicine for running the amino acid analyses in his laboratory and for his assistance in interpreting the results. This investigation was supported by Public Health Service Grant AI-09224-01 from the National Institute of Health.

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