BBA 75027

MULTIPLICITY OF THE AMINO ACID PERMEASES IN *SACCHAROMYCES CEREVISIAE*

III. EVIDENCE FOR A SPECIFIC METHIONINE-TRANSPORTING SYSTEM

J. J. GITS AND M. GRENSON
Laboratoire de Microbiologie, Université de Bruxelles, and Institut de Recherches du C.E.R.I.A., Bruxelles (Belgium)

(Received October 14th, 1966)

SUMMARY

1. The initial rate of entry of L-[^{14}C]methionine into yeast cells appears, on a Lineweaver–Burk plot, to be dependent on two functions. Evidence is presented showing that methionine enters the cell by at least two distinct systems.

2. Mutants which have lost the activity of the methionine permease with a high affinity for methionine (K_m = 12 μM) and retained the methionine permease with low affinity (K_m = 0.77 mM) were isolated and found to be resistant to low doses of L-ethionine.

3. Studies on amino acid uptake in these mutants (met-p_1), as well as competition experiments with the wild-type strain, show that the permease with high affinity for methionine is highly specific.

4. Genetic analysis of the met-p_1 mutation shows that it is recessive, presents a gene-dose effect, and segregates normally as a single gene which is linked neither to arg-p_1 nor to lys-p_1.

5. The residual methionine uptake into a met-p_1 mutant is inhibited by a few structurally similar amino acids only and seems, on this basis, also to be endowed with a narrow specificity.

INTRODUCTION

Two very specific amino acid permeases have been demonstrated in yeast: an arginine permease[1,2] and a lysine permease[3]. However, the isolation of mutants impaired in the transport of all the amino acids[4,5] pointed to the existence of at least one common step in the transport of all the amino acids. The fact that these general mutants were isolated as resistant to DL-ethionine led us to re-examine the methodology of the isolation of these mutants.

If the transport of a given amino acid through the cell membrane involves a specific permease and, in addition, one or more common factor(s), at least two types

of permeability mutations would lead to resistance to a specific metabolic analog of this amino acid: specific permeaseless mutants and mutants with impaired common factor. The simultaneous utilization of two analogs handled by distinct permeases should select this type of general mutants*.

In the same way, if the transport of a given amino acid involves more than one permease, the selection of specific permeaseless mutants should be very difficult since more than one mutation becomes necessary. That this is the case for L-methionine transport in yeast is shown in the present paper.

MATERIALS AND METHODS

These are essentially the same as those described previously[2]. Medium 149 was used exclusively. All the strains (*Saccharomyces cerevisiae*) were isogenic to Σ1278b(α) (wild type); MG168(α)arg-p_1[2], RA382(α)lys-p_1[3], RA276(α)met-p_1, Σ2404b(a) wild type.

The kinetics of entry of all the amino acids tested were linear for at least 2 min following the addition. Samples were taken every 0.5 min for 2 min.

Good reproducibility of results is obtained only when the cells have been maintained in the exponential phase of growth for at least twelve generations. The following values were obtained for six completely independent cultures of Σ1278b. Initial velocity of uptake of 20 μM L-[^{14}C]methionine (in mμmoles per mg protein per min): 8.03; 8.09; 8.07; 8.14; 8.05; 8.08; mean = 8.08.

The labelled amino acids (uniformly ^{14}C-labelled) were obtained from the Radiochemical Centre (Amersham).

RESULTS

Kinetic parameters of L-*methionine uptake in a wild-type strain*

The initial rate of entry of L-[^{14}C]methionine into the wild-type strain Σ1278b appears, on a Lineweaver–Burk plot, as dependent on two functions (Fig. 1). This suggests that L-methionine enters the cells by two distinct systems, one with a high affinity for L-methionine (with K_m of about 12 μM) and the other with a 100-fold lower affinity for L-methionine (with K_m of about 0.77 mM).

Isolation of L-*methionine-permeaseless mutants*

If the methionine analog ethionine is also handled by these two systems, the utilization of high doses of inhibitor would prevent the isolation of specific mutants (since two mutations would be necessary: each affecting one of the systems). In addition, if D-ethionine can be transported by another permease, this would further increase the difficulty of obtaining specific mutants.

On this basis, resistant mutants were selected on a medium containing the lowest dose of L-ethionine capable of inhibiting growth (*e.g.* 0.28 mM). The mutants obtained were tested for resistance to several amino acid analogs: L-canavanine, L-thiosine, β-2-thienylalanine, DL-*p*-fluorophenylalanine and L-ethionine. About 40% of the mutants were resistant to ethionine only. Half of them were of the same type, genetically as well as phenotypically. The prototype of these is RA276 and the muta-

* On the basis of this principle we have selected mutants impaired in the transport of all the amino acids as will be reported elsewhere.

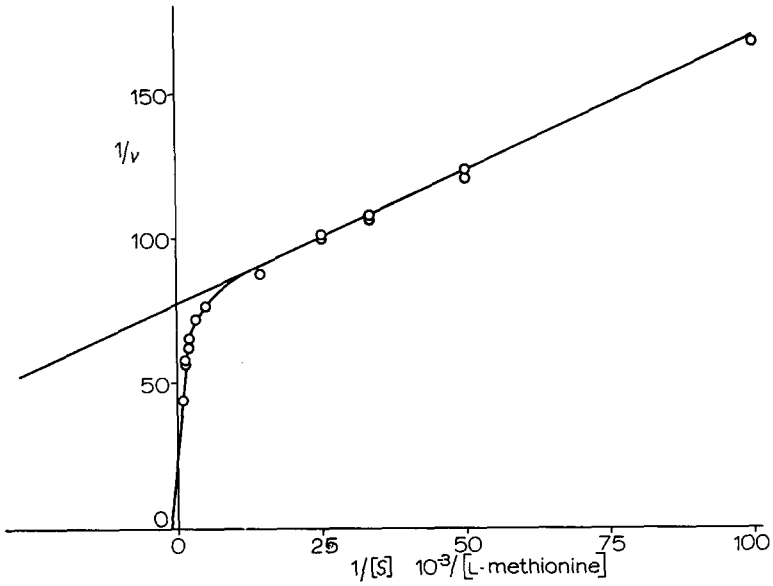

Fig. 1. L-Methionine uptake into a wild-type strain (Σ1278b), v in μmoles per mg protein per min. Experimental data not corrected for overlapping of the two systems. Duplicate points correspond to independent experiments.

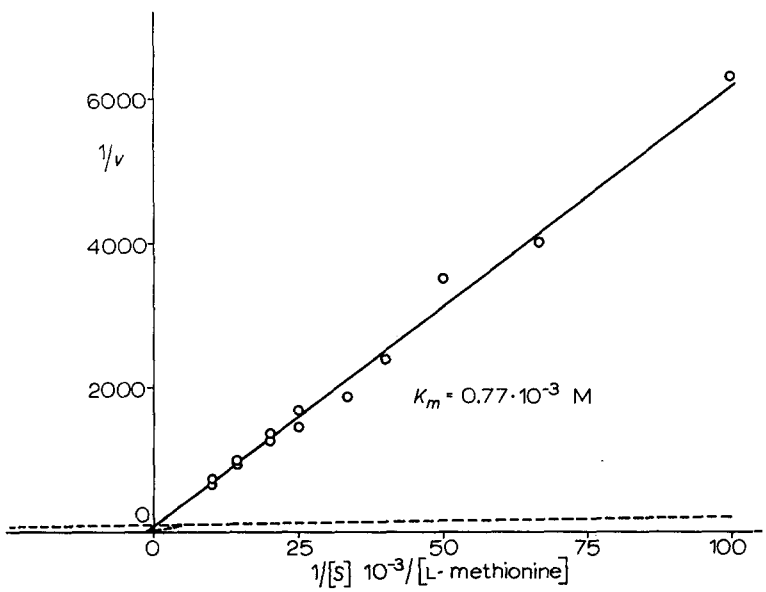

Fig. 2. L-Methionine uptake into a met-p_1 mutant (RA276). v in μmoles per mg protein per min. Duplicate points correspond to independent experiments. For the sake of comparison, the curve of Fig. 1 has been reproduced here (dotted line).

tion has been named met-p_1. None of the met-p_1 mutants were resistant to higher doses of L-ethionine (1.12 mM).

Among the other mutants we found strains with impaired permeability to all the amino acids, corresponding to the phenotype described by SURDIN *et al.*[4] and by SORSOLI, SPENCE AND PARKS[5]. On the basis of these results, which will be presented elsewhere, we conclude that a function involving in the transport of all amino acids into the cells has been affected.

TABLE I

SPECIFICITY OF THE SYSTEM AFFECTED BY THE met-p_1 MUTATION

Initial velocity of entrance of several ^{14}C-labelled amino acids into the mutant RA276 (met-p_1) and the wild-type strain Σ1278b.

Amino acid tested	*Initial velocity of entrance (mμmoles per mg protein per min)*			
	External concentration 0.02 mM		*External concentration 0.2 mM*	
	Σ1278b	*RA276*	*Σ1278b*	*RA276*
L-Methionine	9.0	0.75	13.3	3.6
D-Methionine			3.7*	0.1*
L-Alanine	0.98	1.07	4.25	4.05
L-Arginine	10.85	10.85	12.5	12.5
L-Glycine	0.40	0.42	2.55	2.67
L-Leucine	0.32	0.38	2.07	1.95
L-Isoleucine	0.78	0.82	4.8	4.9
L-Serine	2.5	2.8	20	23.5
L-Threonine	1.5	1.5	14	13.6
L-Valine	0.11	0.12	0.57	0.59

* External concentration: 0.5 mM.

Kinetic parameters of L-methionine uptake in the mutant RA276 (met-p_1)

The Lineweaver–Burk plot for L-methionine uptake obtained with this mutant shows a single function with K_m = 0.77 mM and V = 12.5 mμmoles per mg protein per min (Fig. 2). The activity of the system with high affinity for L-methionine was not detectable.

Specificity of the mutated system: Effect of the mutation on the initial rate of entry of several amino acids into the cell

As it appears in Table I, the mutation affects methionine uptake quite specifically.

Genetic analysis of the met-p_1 mutation

Mendelian monogenic segregation of the met-p_1 mutation. The strain RA276 was crossed with the wild-type isogenic strain Σ2404b and tetrad analysis was carried out by testing the haploid strains for L-ethionine (0.28 mM, medium M) resistance. A normal 2:2 segregation was observed.

Gene-dose effect in the diploid. The heterozygote diploid was compared to both parental type homozygotes. The results are given in Fig. 3, where a gene-dose effect is clearly visible.

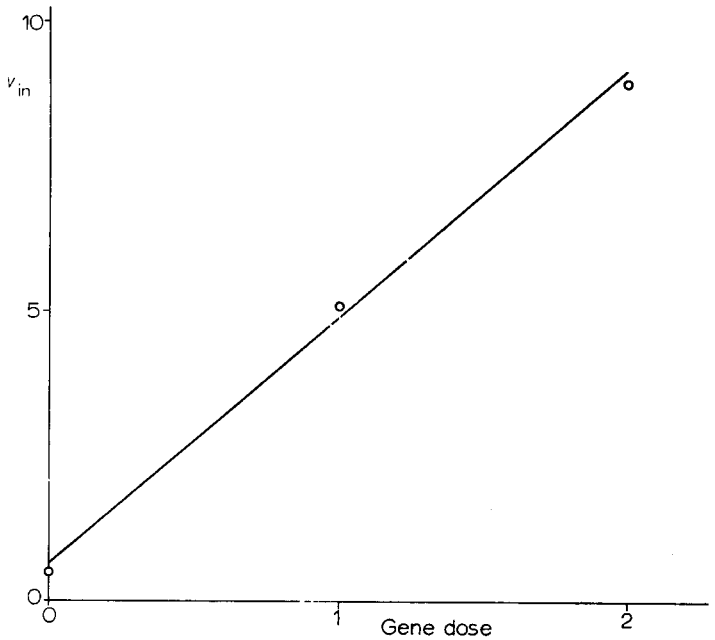

Fig. 3. Gene-dose effect in diploids. v_{in}: initial velocity of entrance of 0.02 mM L-[^{14}C]methionine in mμmoles per mg protein per min. Gene-doses: 0, met-p_1, homozygote; 1, heterozygote; 2, wild-type homozygote.

Independence of the loci met-p_1, arg-p_1 and lys-p_1. The mutant RA276 was crossed with an arg-p_1 mutant. Out of 24 complete tetrads, 3 were parental ditypes, 2 non-parental ditypes and 19 tetratypes.

The same met-p_1 strain was crossed with a lys-p_1 mutant. The tetrads recovered were 1 parental ditype, 2 non-parental ditypes and 6 tetratypes.

The absence of linkage between arg-p_1 and lys-p_1 has been shown previously[3]. Thus, the three permeaseless mutations affect three independent loci.

Dependence of the expression of the met-p_1 mutation on the presence of NH_4^+ in the culture medium

The met-p_1 mutants recover full sensitivity to ethionine as well as normal permeability to methionine (same K_m and V as the wild type) when NH_4^+ is removed from the medium (Fig. 4). The results shown in Fig. 4 were obtained with cells grown with L-proline as the source of nitrogen. The same results are obtained after nitrogen starvation (plateau of a culture on a limiting amount of NH_4^+).

The same type of dependence of the expression of the permease mutation on the presence of NH_4^+ was observed in the case of the arg-p_1 mutation[2] and of the lys-p_1 mutation[3]. The significance of this will be discussed elsewhere.

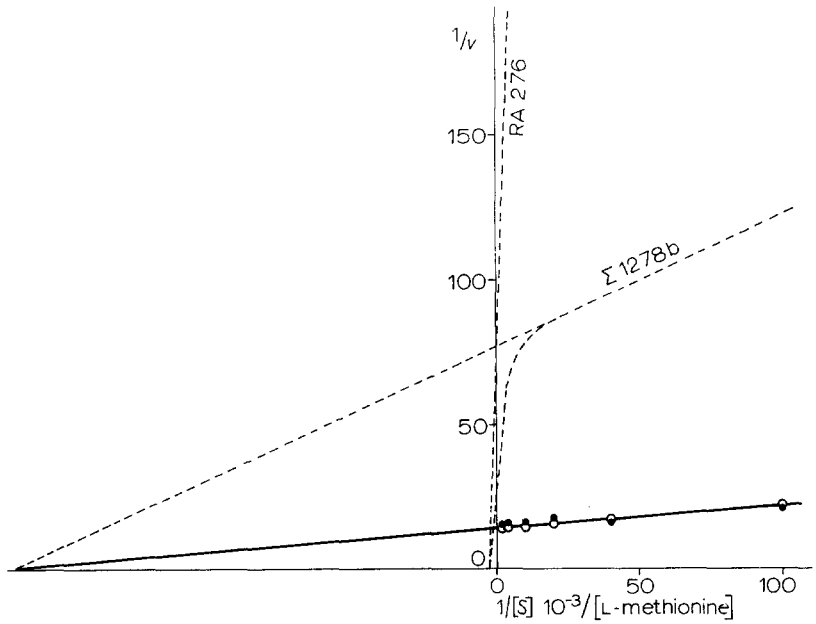

Fig. 4. Effect of the removal of NH_4^+ on the initial velocity of entrance of L-methionine into the wild-type strain Σ1278b and the mutant RA276. Dotted lines: controls on the ammonium medium (data from Figs. 1 and 2). Full lines: cells grown on L-proline as source of nitrogen; ●—●, RA 276; ○—○, Σ1278b.

Specificity of the permease with high affinity for methionine as determined by competition experiments with the wild-type strain

The initial rate of entry of 0.02 mM L-[^{14}C]methionine was measured in the presence of other, unlabelled, amino acids at a 10-fold higher concentration. In the presence of this concentration of methionine, the activity of the uptake system with low affinity for methionine is negligible.

The results given in Table II show the high specificity of the permease with high affinity for methionine. A significant inhibition was observed only with closely related substances like L-ethionine, DL-selenomethionine, and D-methionine. This inhibition is competitive and the apparent affinity constants of the uptake system with high affinity for methionine for the inhibiting substances have been estimated on the basis of the results given in Figs. 5–7.

It has been shown that the small inhibition by arginine for instance is exerted at another level than the inhibition by molecules which are structurally more similar to methionine. First, it is limited and, thus, not competitive. Secondly, arginine does not inhibit methionine uptake in an arginine-permeaseless strain (MG168), which shows that arginine does not act directly at the level of the methionine permease but has to react with its own permease in order to inhibit methionine uptake.

Specificity of the residual methionine uptake system in a met-p_1 mutant (RA276)

A preliminary study of this system by competition experiments (Table III) shows that it is not inhibited by a number of amino acids present at a 10-fold higher

TABLE II

SPECIFICITY OF THE PERMEASE WITH HIGH AFFINITY FOR METHIONINE

Competition experiments with the wild-type strain Σ1278b.

Unlabelled amino acid added (0.2 mM)	*Initial velocity of entrance of 0.02 mM* L-[^{14}C]*methionine (mμmoles per mg protein per min)*
—	8.08
L-Alanine	7.90
L-Arginine	7.30
L-Cysteine	7.97
DL-Homocysteine*	7.66
D-Ethionine	7.50
L-Glutamate	8.04
Glycine	8.03
DL-Homoserine*	7.40
L-Leucine	7.30
L-Isoleucine	8.04
L-Cycloleucine	8.10
L-Norleucine	8.04
L-Proline	8.02
L-Serine	7.45
L-Threonine	7.55
L-Valine	8.01
L-Norvaline	8.06
D-Methionine	6.40
L-Ethionine	4.66
DL-Selenomethionine	2.2

* External concentration: 0.4 mM (DL).

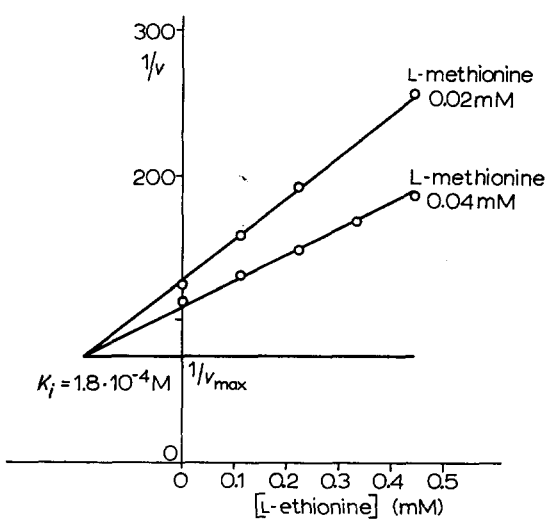

Fig. 5. Competitive inhibition of L-methionine uptake by L-ethionine. Strain Σ1278b. *v* in μmoles per mg protein per min.

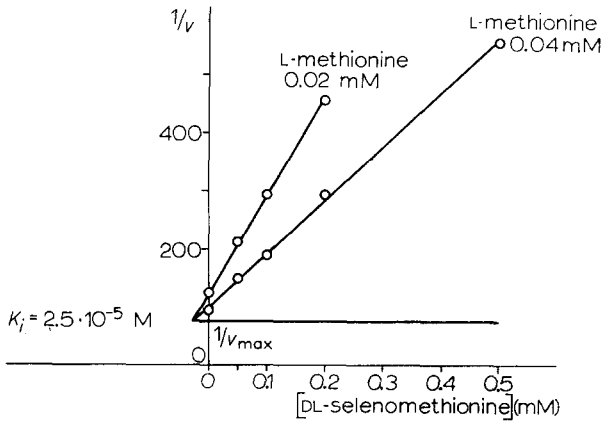

Fig. 6. Competitive inhibition of L-methionine uptake by DL-selenomethionine. Strain Σ1278b. v in μmoles per mg protein per min.

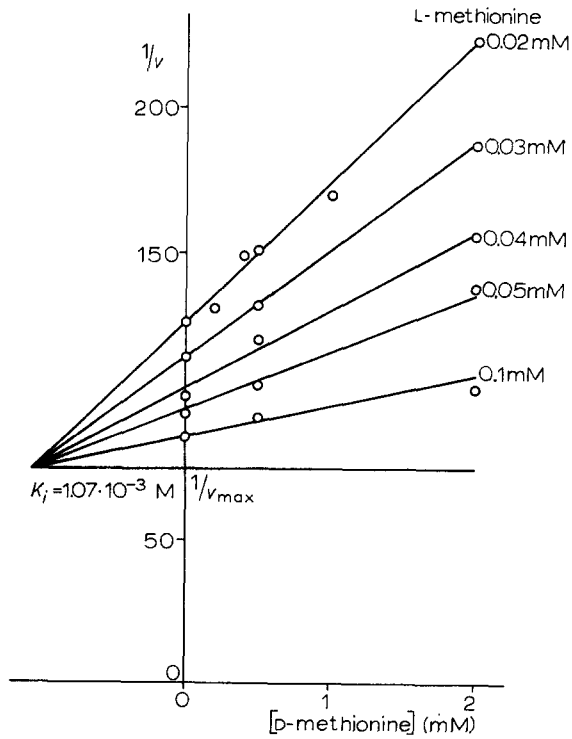

Fig. 7. Competitive inhibition of L-methionine uptake by D-methionine. Strain Σ1278b. v in μmoles per mg protein per min.

TABLE III

SPECIFICITY OF THE RESIDUAL METHIONINE UPTAKE SYSTEM IN A met-p_1 MUTANT (RA276)

Initial velocity of entrance of L-[^{14}C]methionine (0.05 mM) in the presence of several unlabelled amino acids (0.5 mM).

Amino acid added	*Initial velocity of entrance of L-[^{14}C]-methionine (mμmoles per mg protein per min)*
—	0.80
L-Alanine	0.74
L-Arginine	0.66
L-Cysteine	0.73
DL-Homocysteine (1 mM)	0.92
D-Ethionine	0.84
L-Glutamate	0.80
Glycine	0.78
L-Leucine	0.72
L-Isoleucine	0.73
D-Methionine	0.74
L-Proline	0.76
L-Valine	0.81
L-Threonine	0.60
L-Ethionine	0.54
DL-Selenomethionine (1 mM)	0.59
L-Serine	0.60

concentration than methionine. Only L-ethionine, DL-selenomethionine, L-threonine and L-serine are inhibitory.

DISCUSSION AND CONCLUSIONS

The existence of two separate mechanisms of uptake for methionine is easily detectable even in the wild-type strain due to the fact that the permease with high affinity for methionine has a lower V than the system with low affinity for methionine. Obviously, systems which do not have this characteristic would be masked by the other(s).

The specificity of the permease with high affinity for methionine (as measured by competition experiments as well as by the properties of a met-p_1 mutant) allows us to consider this system as the methionine permease.

The specificity of the system with low affinity for methionine does not seem to be much lower than that of the methionine permease. It might correspond to a serine permease or a threonine permease, or both, in the same way as the uptake system with low affinity for lysine has been shown to be the arginine permease[3]. This situation can also to compared with the case of histidine uptake by *Escherichia coli* (see ref. 6) where two systems are also present, one very specific, with high affinity for histidine, and the other with lower affinity but higher V, which could be defined as a permease transporting all the aromatic amino acids in addition to histidine.

Although important differences in the experimental conditions* do not allow direct comparison of the results, the observations of MAW[7] on the uptake of sulphur-containing amino acids by brewer's yeast are in agreement with ours in showing competitive effects restricted to compounds which are closely related in structure to a given sulphur amino acid.

ACKNOWLEDGEMENTS

This investigation was supported by a research grant from the Fonds de la Recherche Scientifique Fondamentale Collective (Belgium).

The technical assistance of Mrs. F. VERHAEGEN-MUYLDERMANS is gratefully acknowledged.

J. J. G. is greatful for a grant from IRSIA (Institut pour l'encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture).

REFERENCES

1 J. M. WIAME, J. BECHET, M. MOUSSET AND M. GRENSON, *Arch. Intern. Physiol. Biochim.*, 70 (1962) 766.
2 M. GRENSON, M. MOUSSET, J. M. WIAME AND J. BECHET, *Biochim. Biophys. Acta*, 127 (1966) 325.
3 M. GRENSON, *Biochim. Biophys. Acta*, 127 (1966) 339.
4 Y. SURDIN, W. SLY, J. SIRE, A. M. BORDES AND H. DE ROBICHON-SZULMAJSTER, *Biochim. Biophys. Acta*, 107 (1965) 546.
5 W. A. SORSOLI, K. D. SPENCE AND L. W. PARKS, *J. Bacteriol.*, 88 (1964) 20.
6 G. FERROLUZZI-AMES, *Arch. Biochem.*, 104 (1964) 1.
7 G. A. MAW, *J. Gen. Microbiol.*, 31 (1963) 247.

* The principal difference is that we measure the initial rate of entry of the labelled amino acid for 2 min after addition while in MAW's experiments the first measure is made after 30 min.