JOURNAL OF BACTERIOLOGY, Nov. 1981, p. 514-520
0021-9193/81/110514-07$02.00/0

Vol. 148, No. 2

Methionine Sulfoxide Is Transported by High-Affinity Methionine and Glutamine Transport Systems in *Salmonella typhimurium*

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Received 4 June 1981/Accepted 28 July 1981

Three lines of evidence indicated that methionine sulfoxide is transported by the high-affinity methionine and glutamine transport systems in *Salmonella typhimurium*. First, methionine-requiring strains (*metE*) which have mutations affecting both of these transport systems (*metP glnP*) were unable to use methionine sulfoxide as a source of methionine. These strains could still grow on L-methionine because they possessed a low-affinity system (or systems) which transported L-methionine but not the sulfoxide. A methionine auxotroph with a defect only in the *metP* system, which was dependent upon the *glnP*$^+$ system for the transport of methionine sulfoxide, was inhibited by L-glutamine because glutamine inhibited the transport of the sulfoxide by the *glnP*$^+$ system. Second, a *metE metP glnP* strain could be transduced at either the *metP* or *glnP* genes to restore its ability to grow on methionine sulfoxide. Third, the transport of [^{14}C]methionine sulfoxide was inhibited by methionine and by glutamine in the *metP*$^+$ *glnP*$^+$ strain. No transport was detected in the *metP glnP* double-mutant strain.

Methionine sulfoxide is a source of methionine for methionine auxotrophs in *Escherichia coli* (7, 8, 12). A complex enzyme system which brings about the reduction of free methionine sulfoxide has been described in yeast cells (4, 11) and more recently in *E. coli* (8). Since methionine sulfoxide cannot be attached to tRNA^{Met} (9), it seems likely that the sulfoxide is first transported into the cell and is then reduced to methionine before it is incorporated into protein or used as a source of methyl groups.

The methionine sulfoxide-reducing system in yeast cells consisted of three proteins: thioredoxin, thioredoxin reductase, and methionine sulfoxide reductase (11). The first two proteins served to generate NADPH and were thus nonspecific, whereas the methionine sulfoxide reductase was specific for this substrate. It was originally suggested that the real substrate might be methionine sulfoxide residues in protein (6). Oxidation of methionine residues in proteins may occur in cells by the action of reagents such as hydrogen peroxide. This reaction has been shown to result in vitro in the loss of biological activity in several proteins, for example, ribosomal protein L12 (6). Such oxidations may occur in vivo, and the presence of a relatively large amount of methionine sulfoxide in human cataractous lenses proteins has been reported (18). Thus, the ability of cells to maintain methionine in its reduced state may be an important cellular activity.

However, it is now clear that there are two distinct enzymes with methionine sulfoxide reductase activities. One enzyme is specific for methionine sulfoxide residues in proteins (5), and the other is specific for free methionine sulfoxide (9). The biological role of the second enzyme is not yet clear.

Little was known about the mechanism of transport of methionine sulfoxide in bacteria, except that sulfoxide was a poor inhibitor of methionine transport (1, 12, 14). Starting from the observation that methionine sulfoxide supported the growth of methionine auxotrophs in *Salmonella typhimurium*, I investigated whether the sulfoxide was transported by one of the methionine transport systems or whether it entered the cell by a different route. Methionine itself is transported by at least two systems. First, there is a high-affinity system (apparent K_m, about 0.1 μM) which has a reduced activity or is missing in *metP* mutants (1, 2). Second, there is one system, or possibly two systems, with relatively low affinities for methionine (2), but mutants defective in these systems have not been isolated.

One selection procedure used to isolate mutants defective in the high-affinity methionine transport system was to select for resistance to

the potent growth inhibitor methionine sulfoximine (1, 3). One class of mutants resistant to this inhibitor contained two mutations, one in the *metP* gene and the other in the *glnP* gene (3). *glnP*$^+$ specifies a component of the high-affinity glutamine transport system (3). Thus, it appeared that methionine sulfoximine is transported by two different systems, and both systems had to be blocked to bring about full resistance to this analog.

The effect of the *metP* or *glnP* mutation on the ability of methionine auxotrophs to grow on methionine sulfoxide was determined. Only strains lacking both transport systems were unable to grow. Therefore, it was concluded that methionine sulfoxide, similarly to methionine sulfoximine, is transported by both the *metP* and *glnP* high-affinity transport systems. This interpretation was confirmed by the failure of the *metP glnP* mutants to transport methionine sulfoxide in an assay for uptake activity.

MATERIALS AND METHODS

Strains. The genotypes of most of the strains used in this work are given in Table 1. In addition, *metA43 purE11* (HU29), *metB23* (HU33), *metC819* (HU505), *metF185* (HU43), *metG319* (HU299), *metG419* (HU300), and *metE205 metH463 ara-9* (HU48) were from the laboratory collection and were originally obtained from D. A. Smith. *metE* mutants are unique among methionine auxotrophs in that they respond to methionine or vitamin B_{12} (cyanocobalamin) (17).

Media. Nutrient agar (code CM3) and nutrient broth (code CM1) were supplied by Oxoid Ltd. Minimal medium contained the following (in grams per liter): K_2HPO_4, 10.5; KH_2PO_4, 4.5; trisodium citrate·$2H_2O$, 0.47; $(NH_4)_2SO_4$, 1; $MgSO_4 \cdot 7H_2O$, 0.05; and D-glucose, 4. Minimal agar was minimal medium containing 0.2%, rather than 0.4%, glucose, solidified with 1.5% New Zealand agar (British Drug Houses, Ltd.). Arabinose and galactose minimal agar, for the selection of Ara$^+$ and Gal$^+$ phenotypes in transduction crosses, contained 1% L-arabinose or 1% D-galactose, respectively, and lacked glucose and sodium citrate. L-Methionine, D-methionine, L-methionine sulfone (Sigma Chemical Co.), and L-methionine-DL-sulfoxide (Koch-Light Laboratories, Ltd.) were added to media at 20 $\mu g\ ml^{-1}$. Solutions of L-glutamine (Sigma Chemical Co.) were freshly prepared and added to the media at 100 $\mu g\ ml^{-1}$. Vitamin B_{12} (British Drug Houses, Ltd.) was added at 0.1 $\mu g\ ml^{-1}$.

Chemicals. L-[*methyl*-^{14}C]methionine (60.2 mCi $mmol^{-1}$, 2.23 GBq $mmol^{-1}$) was obtained from the Radiochemical Centre. L-[^{14}C]methionine-DL-sulfoxide was prepared by incubating 0.42 mM L-[*methyl*-^{14}C]methionine for 2 h at room temperature with 0.3 or 1.6% (wt/vol) hydrogen peroxide in 1 ml of phosphate buffer (pH 7.0). The mixture was freeze-dried and suspended in 0.2 ml of water. It was then spread across a 0.5-mm-thick cellulose thin-layer chromatography plate, and the plate was run in *tert*-butyl alcohol–methyl ethyl ketone–water (2:2:1 [vol/vol/vol]) containing 4 ml of diethylamine per 100 ml of solvent. The radioactive areas were located with a Panax thin-layer scanner and were identified by comparison with nonradioactive standards which had been run at the edges of the plate. The [^{14}C]methionine sulfoxide spot was scraped off and eluted into 3 ml of minimal medium without glucose. The cellulose was removed by centrifugation, and the solution was filter sterilized. The purity of the [^{14}C]methionine sulfoxide was determined by thin-layer chromatography in *tert*-butyl alcohol–methyl ethyl ketone–water (2:2:1 [vol/vol/vol], with 4 ml of diethylamine added to each 100 ml of solvent) and phenol-water (4:1 [vol/vol]). No L-methionine was detected in the solution in system 1 (methionine and methionine sulfoxide run close together in system 2 and thus cannot be distinguished). The solution contained approximately 10% L-methionine sulfone in systems 1 and 2.

Growth of cultures. For testing of responses on solid media, bacteria were grown overnight in 0.5 ml of nutrient broth and suspended in 2 ml of 0.85% saline. The suspensions were then streaked onto minimal agar plus the indicated supplements. For experiments with *metE* strains in supplemented minimal medium, bacteria were grown overnight in 10 ml of minimal medium plus L-methionine at 20 $\mu g\ ml^{-1}$, with the glucose concentration reduced to 0.02%. The next morning, glucose was added at 0.4%, and bacteria were grown for 75 min. The cultures were centrifuged and suspended in 2 ml of minimal medium without glucose. The suspensions were then used to inoculate the experimental flasks. For the transport assays involving *metE* strains, bacteria were grown in 50 ml of minimal medium plus vitamin B_{12} at 0.1 $\mu g\ ml^{-1}$. Vitamin B_{12} was used, since, unlike L-methionine, it does not result in the repression of the *metP*$^+$ transport system (2; unpublished data). After centrifugation, the cultures were washed with a culture volume of minimal medium plus chloramphenicol at 200 $\mu g\ ml^{-1}$ and resuspended in 5 ml of the same medium. The suspensions were then adjusted to 2 to 4 mg (dry weight) ml^{-1} and kept at 25°C.

Transport assay. The transport assay was performed essentially as previously described (2). [^{14}C]-methionine sulfoxide with or without unlabeled L-methionine or L-glutamine was incubated for at least 2 min at 25°C. The assay was initiated by the addition of bacteria. Samples were taken at 30 s and filtered through 0.45-μm-pore-size filters (Oxoid Ltd.). The filters were washed once with 5 ml of minimal medium with chloramphenicol, dried, placed in 5 ml of scintillation fluid (2), and counted at 80% efficiency in an Intertechnique scintillation counter.

Transduction. Transduction was performed with phage P22 HT *int-4*. For strain construction, lysates were prepared by adding 10^6 phage to the donor bacteria in soft nutrient agar layers, and transductions were performed directly on the minimal agar surface (10); 0.05 ml of a donor phage lysate and 0.05 ml of an overnight nutrient broth culture of the recipient bacteria were mixed and spread on selective minimal agar. For other transductions, phage were prepared by two cycles of lysis on the donor bacterial culture: they were first propagated on HU471, HU470, HU469, and HU468 in soft nutrient agar layers. Samples of these

TABLE 1. *Bacterial strains*

Strain	Genotype	Origin or reference
HU103	LT2 wild type	Laboratory collection
HU36	*metE205 ara-9*	Laboratory collection
HU439	*metE205*	HU36[a] transduction with HU103 as donor
HU425	*metE205 metP760*	HU163 transduction with HU103 as donor
HU478[b]	*metE205 metP761 glnP251*	HU426 transduction with HU103 as donor
HU479[b]	*metE205 metP762 glnP253*	HU427 transduction with HU103 as donor
HU428	*metE205 metP763*	HU181[a] transduction with HU103 as donor
HU429	*metE205 metP764*	HU182[a] transduction with HU103 as donor
HU430[b]	*metE205 metP765*	HU108[a] transduction with HU103 as donor
HU431	*metE205 metP766*	HU109[a] transduction with HU103 as donor
HU468	*metE205 metP767 glnP252*	HU262 transduction with HU103 as donor
HU159	*metE205 metP768 glnP254*	HU142 conjugation with HU36 as recipient
HU433	*metE205 metP1707*	HU253[a] transduction with HU103 as donor
HU434	*metE205 metP1708 glnP256*	HU349[a] transduction with HU103 as donor
HU435	*metE205 metP1709 glnP257*	HU350[a] transduction with HU103 as donor
HU436	*metE205 metP1710 glnP258*	HU412[a] transduction with HU103 as donor
HU437	*metE205 metP1711 glnP259*	HU413[a] transduction with HU103 as donor
HU438	*metE205 metP1712 glnP260*	HU414[a] transduction with HU103 as donor
HU163	*metE205 metP760 ara-9*[c]	3
HU426	*metE205 metP761 glnP251 galK50*	HU84 transduction with HU103 as donor
HU84	*metE205 metP761 glnP251 ara-9*[c] *galK50*[c]	3
HU427	*metE205 metP762 glnP253 galK50*	HU421 transduction with HU36 as donor
HU421	*metE205 metP762 glnP253 leu galK50*	HU151 mutagenesis with NG[d]
HU151	*metE205 metP762 glnP253 galK50*[c]	2
HU262	*metE205 metP767 glnP252 galK50*[c]	3
HU142	HfrK2 *hisD23 metP768 glnP254*	HU18 spontaneous MS[r] mutant[e]
HU18	HfrK2 *hisD23*	K. E. Sanderson
HU424	*metE205 metP767 glnP252 ara-9 galK50*	HU423 transduction with HU36 as donor
HU423	*metE205 metP767 glnP252 leu galK50*	HU262 mutagenesis with NG
HU469	*metE205 metP767 glnP*$^+$	HU468 transduction with HU36 as donor
HU470	*metE205 metP*$^+$ *glnP252*	HU468 transduction with HU36 as donor
HU471	*metE205 metP*$^+$ *glnP*$^+$	HU469 transduction with HU36 as donor

[a] These strains are all *ara-9* and are fully described in reference 2.
[b] HU478, HU479, and HU430 were constructed by A. Cottam.
[c] These mutations were not listed in the genotypes given in reference 2.
[d] NG, *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine.
[e] MS[r], Resistance to L-methionine-DL-sulfoximine at 50 μg ml^{-1}.

lysates (10^8 phage) were then added to 50-ml log-phase cultures of the same recipients, and the phage were repropagated (16). The resulting titers were between 6×10^{10} and 10×10^{10} phage ml^{-1}. Transductions with these phage preparations were performed by preincubating phage and bacteria for 10 min at 37°C before samples were spread on minimal agar plus methionine sulfoxide.

RESULTS

Mutants unable to use methionine sulfoxide. Representatives of all of the known classes of methionine auxotrophs in *S. typhimurium* (17) were able to use methionine sulfoxide but not methionine sulfone (*metA43, metB23, metC819, metE205, metF185, metG319, metG419,* and *metE205 metH463*). For *metE205* (strain HU471), both the rate of growth and the final growth yield in liquid minimal medium plus L-methionine sulfoxide at 20 μg ml^{-1} were similar to those on L-methionine at 20 μg ml^{-1} (Fig. 1).

The effect of various *metP* and *glnP* mutations on the ability of *metE205* to grow on methionine sulfoxide was determined (Table 2). HU439, the control strain carrying the *metE205* mutation, grew well on D-methionine, L-methionine, methionine sulfoxide, and vitamin B_{12}, as expected. All of the *metP*-containing strains, with the exception of HU435 (*metE205 metP1709 glnP257*) and HU438 (*metE205 metP1712 glnP260*), failed completely to grow on D-methionine. HU435 and HU438 showed only partial growth on D-methionine; the precise amount varied from test to test and was very dependent on the inoculum size. The tests on methionine sulfoxide showed that seven of the nine *metE metP glnP* strains failed to grow on this compound; only HU435 and HU438 gave positive results. Of the six strains containing only the *metP* mutation, all but one grew on methionine sulfoxide. HU430 (*metE205 metP765*) showed only a variable amount of poor growth on methionine sulfoxide; it was an unusual strain in that, for unknown reasons, it grew poorly on L-methionine and not at all on vitamin

B_{12}. Thus, its poor growth on methionine sulfoxide was probably due to the poor use of the methionine derived from methionine sulfoxide, rather than from a direct defect in the use of methionine sulfoxide.

Failure to grow on methionine sulfoxide requires *metP* and *glnP* mutations. The above results suggested three possible reasons for the failure of the *metE metP glnP* strains to grow on methionine sulfoxide: (i) methionine sulfoxide behaved as an analog of both methionine and glutamine and thus entered the bacterium via the $metP^+$ and $glnP^+$ transport systems; (ii) methionine sulfoxide entered only via the $glnP^+$ system; and (iii) methionine sulfoxide entered via the $metP^+$ system, but only those *metP* mutations in the *metP glnP* strains abolished uptake of this compound. If methionine sulfoxide were transported by both the $metP^+$ and $glnP^+$ systems at a rate sufficient to sustain growth, then it should be possible to restore the ability of the *metE metP glnP* strains to grow on methionine sulfoxide by transducing the *metP* or *glnP* mutations out of the strains. Strain HU468 (*metE205 metP767 glnP252*) was transduced with donor phage grown on strain HU36 (*metE205 ara-9*) on minimal agar plus methionine sulfoxide. Approximately 95% of the transductants were also able to grow on minimal agar plus D-methionine and were therefore of the genotype *metE205* $metP^+$ *glnP252*; one of these transductants was retained as HU470. The other 5% of the transductants failed to grow on D-methionine and were therefore *metE205 metP767* $glnP^+$; a typical transductant was kept as HU469. Finally, HU47 (*metE205* $metP^+$ $glnP^+$) was derived by transducing HU469 on minimal agar plus D-methionine with donor

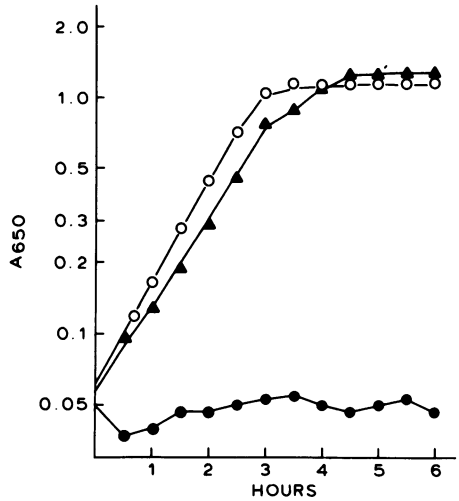

FIG. 1. *Growth of the metE205 methionine auxotroph (HU471) with* L*-methionine or* L*-methionine-*DL*-sulfoxide. Symbols:* ○, *20 μg of* L*-methionine* ml^{-1}; ▲, *20 μg of* L*-methionine-*DL*-sulfoxide* ml^{-1}; ●, *no addition.* A_{650}, *Absorbancy at 650 nm.*

TABLE 2. *Effects of metP and glnP mutations on the growth of the metE205 auxotroph on methionine sulfoxide*[a]

Strain	General genotype	Growth on minimal agar plus:				
		D-Methionine, (20 μg ml^{-1})	L-Methionine sulfoxide, (20 μg ml^{-1})	L-Methionine sulfoxide + L-glutamine (100 μg ml^{-1})	Vitamin B_{12} (0.1 μg ml^{-1})	L-Methionine (20 μg ml^{-1})
HU439	*metE205* $metP^+$ $glnP^+$	+	+	+	+	+
HU425, HU428, HU429, HU431, HU433	*metE205 metP* $glnP^+$	−	+	−	+	+
HU430	*metE205 metP* $glnP^+$	−	±	−	−	±
HU478, HU479, HU468, HU159, HU434, HU436, HU437	*metE205 metP glnP*	−	−	−	+	+
HU435, HU438	*metE205 metP glnP*	±	+	+	+	+

[a] Saline suspensions were streaked onto various media, and the results were scored after 24 h. +, Full growth response; ±, intermediate growth response; −, no growth.

TABLE 3. *Growth of metE metP glnP recombinants on methionine sulfoxide*[a]

Strain	Genotype	Growth on minimal agar plus:		
		D-Methionine	L-Methionine sulfoxide	L-Methionine sulfoxide + L-glutamine
HU471	*metE205 metP*$^+$ *glnP*$^+$	+	+	+
HU470	*metE205 metP*$^+$ *glnP252*	+	+	+
HU469	*metE205 metP767 glnP*$^+$	−	+	−
HU468	*metE205 metP767 glnP252*	−	−	−

[a] Details as in Table 2.

phage grown on HU36. Thus, HU471, HU470, HU469, and HU468 are isogenic apart from the *metP* and *glnP* mutations, and their behavior on D-methionine and methionine sulfoxide is summarized in Table 3. The genotypes of these strains were also directly confirmed by assays of methionine and glutamine transport activities (data not shown). It was therefore clear from these results that methionine sulfoxide was transported by both the *metP*$^+$ and *glnP*$^+$ transport systems and that the activity of either system was sufficient to maintain growth.

Inhibition of growth on methionine sulfoxide by glutamine. Since glutamine is presumably the natural substrate of the *glnP*$^+$ system, it seemed likely that glutamine would inhibit the uptake of methionine sulfoxide by this system. Thus, it might inhibit the growth of HU469 (*metE205 metP767 glnP*$^+$) on methionine sulfoxide. This indeed turned out to be the case (Table 3). Of the three strains growing on methionine sulfoxide, i.e., HU471, HU470, and HU469, only HU469 was inhibited by L-glutamine at 100 μg ml^{-1}. In HU471 and HU470, methionine sulfoxide could enter via the *metP*$^+$ system. Glutamine was also shown to inhibit the growth of those strains (Table 2) which were mutated only in *metP* but not in *glnP* (those strains which were of the general genotype *metP glnP*$^+$ [HU425, HU428, HU429, HU430, HU431, and HU433]).

The results in Table 3 were confirmed by tests in liquid minimal medium (data not shown). HU471, HU470, HU469, and HU468 all grew on L-methionine with a doubling time of 39 to 46 min; all strains except HU468 grew on methionine sulfoxide with similar doubling times and final growth yields. Glutamine at 100 μg ml^{-1} completely inhibited the growth of HU469, but not of HU470 or HU471. With methionine sulfoxide reduced to 5 μg ml^{-1} and L-glutamine at 5 mg ml^{-1}, the growth of HU470 and HU471 was partially inhibited.

Genetic analysis of strains unable to grow on methionine sulfoxide. Phage propagated on strains HU471, HU470, HU469, and HU468 were used to transduce the recipient strain HU424 (*metE205 metP767 glnP252 ara-9 galK50*) on methionine sulfoxide (Table 4). Donor phage HU471 generated transductants of two genotypes, *metP*$^+$ *glnP252* and *metP767 glnP*$^+$. HU470 generated only *metP*$^+$ *glnP252* transductants, and HU469 produced only *metP767 glnP*$^+$ transductants. Donor phage HU468 was not able to produce transductants of either class, although, similarly to phages HU471, HU470, and HU469, it was able to transduce the *ara* mutation in HU424. No double *metP*$^+$ *glnP*$^+$ transductants would be expected from phage HU471, since the *metP* and *glnP* genes are thought to be several map units apart (15). This analysis further confirmed that restoration of activity of either the *metP* or *glnP* transport system was sufficient to allow growth on methionine sulfoxide.

Transport of methionine sulfoxide. The transport of L-[^{14}C]methionine-DL-sulfoxide and the effect of an excess of unlabeled L-methionine or L-glutamine were directly assayed in strains HU471, HU470, HU469, and HU468 (Table 5). The activity in HU470 (which lacked the *glnP*$^+$ system) was about 83% of that in HU471, whereas the activity in HU469 (which lacked the *metP*$^+$ system) was about 4% of that in HU471; thus, the major route of entry was deduced to be through the *metP*$^+$ system. HU468 showed almost no activity, thus giving direct evidence for the idea previously suggested that methionine sulfoxide is transported by both the *metP* and *glnP* systems. The effect of a 100-fold excess of unlabeled methionine on methionine sulfoxide transport confirmed these conclusions. Thus, the transport in HU471 was reduced to about 1%, that in HU470 was virtually abolished, and the low level of activity in HU469 (due to the *glnP*$^+$ system) was hardly affected. A 100-fold excess of L-glutamine reduced the transport in HU471 to 37% of the control value. Interestingly, glutamine also inhibited the transport in HU470 to about the same degree as in HU471. A 1,000-fold excess of glutamine reduced sulfoxide transport in HU470 even further, to about 7%. It should be noted that the concentration of methionine sulfoxide in this assay was 0.7 μM compared with 120 μM (20 μg ml^{-1}) in the growth medium. Since the apparent K_m of methionine

TABLE 4. *Transduction of metE205 metP767 glnP252 ara-9 (HU424) on methionine sulfoxide*[a]

Strain	Donor phage genotype[b]	No. of transductants on:		% of transductants on methionine sulfoxide which were[e]:	
		Arabinose + vitamin B_{12}[c]	Methionine sulfoxide[d]	*metP*$^+$ *gln252*	*metP767 glnP*$^+$
HU471	*metE205 metP*$^+$ *glnP*$^+$	180	189	96	4
HU470	*metE205 metP*$^+$ *glnP252*	246	147	100	0
HU469	*metE205 metP767 glnP*$^+$	111	7	0	100
HU468	*metE205 metP767 glnP252*	50	0		

[a] A total of 0.5 ml of phage (10^{10} phage) was mixed with 0.5 ml of recipient bacteria and incubated for 10 min at 37°C, and 0.1-ml samples were spread onto each medium in duplicate.
[b] Phage were prepared by two cycles of lysis on the donor strains.
[c] Minimal agar with arabinose as the carbon source plus vitamin B_{12}.
[d] Minimal agar plus L-methionine-DL-sulfoxide.
[e] The two classes were identified by streaking 100 transductants from each cross performed on L-methionine sulfoxide to a fresh L-methionine sulfoxide plate. They were then restreaked on D-methionine or L-methionine sulfoxide plus glutamine; *metP*$^+$ *glnP252* transductants grew on both media, and *metP767 glnP*$^+$ transductants failed to grow on both media (as in Table 3). (Additional plates of L-methionine sulfoxide involving phage HU469 were prepared to obtain 100 transductants.)

TABLE 5. *Transport of methionine sulfoxide*[a]

Strain	Genotype	Uptake[b]		
		No addition	L-Methionine[c]	L-Glutamine[c]
HU471	*metE205 metP*$^+$ *glnP*$^+$	100	1.1	36.9
HU470	*metE205 metP*$^+$ *glnP252*	82.9	0.1	39.1 6.8[d]
HU469	*metE205 metP767 glnP*$^+$	3.9	3.4	0.1
HU468	*metE205 metP767 glnP252*	0.1	ND[e]	ND

[a] Bacteria were grown in minimal medium plus vitamin B_{12}.
[b] Uptake of 0.70 μM L-[^{14}C]methionine sulfoxide expressed as a percentage of the uptake by strain HU471 of methionine sulfoxide alone (0.98 nmol min^{-1} mg^{-1} [dry wt]).
[c] 70 μM L-methionine or 70 μM L-glutamine included in the assay.
[d] 700 μM L-glutamine.
[e] ND, Not determined.

sulfoxide for the *metP*$^+$ and *glnP*$^+$ systems has not been determined, it is possible that the *glnP*$^+$ system may be more active at the higher concentration.

DISCUSSION

These experiments directly demonstrated that methionine sulfoxide is transported in *S. typhimurium* by the high-affinity methionine and glutamine transport systems. Thus, methionine sulfoxide behaves as an analog of methionine and glutamine at the level of entry into the cell and is, in this respect, similar to the growth inhibitory analog methionine sulfoximine (3). Of nine *metE metP glnP* mutants tested in Table 2, seven failed to grow on methionine sulfoxide. Two strains, HU435 (*metE205 metP1709 glnP257*) and HU438 (*metE205 metP1712 glnP260*), which did grow on methionine sulfoxide, also grew at a reduced rate on D-methionine. This suggested that the activity of the *metP* system in these strains was only reduced, rather than completely abolished. Previous results had indicated that *metP1709* resulted in only a partial loss of methionine transport activity, whereas *metP1712* resulted in a complete loss of methionine transport activity (2). However, HU435 and HU438 have been reexamined for methionine transport and have been shown to possess about 16 and 40%, respectively, of the activity in the wild-type strain (unpublished data). Thus, there is no discrepancy between methionine transport activity and growth characteristics in these strains, although it is not known why the effect of *metP1712* is now much less severe.

The inhibition by glutamine of the growth on methionine sulfoxide of strains of the general genotype *metE metP glnP*$^+$ was entirely consistent with the idea of a dual route of entry for methionine sulfoxide, because such strains depend on the activity of the *glnP*$^+$ system for growth. That the inhibition was at the level of entry into the cell was directly confirmed by assays of transport activity. Thus, transport in HU469 (*metE205 metP767 glnP*$^+$) was completely abolished by glutamine, but not affected by methionine. The inhibition by glutamine of

methionine sulfoxide transport by the *metP*$^+$ system in strain HU470 raised the possibility that this system, or a component of this system, might function in the transport of glutamine. A similar suggestion has previously been made from studies on the inhibition by glutamine of D-methionine transport by the *metP*$^+$ system (J. Poland and P. D. Ayling, Heredity **45**:147, 1980).

If this suggestion were true, then glutamine should inhibit the growth of HU471 and HU470. This was indeed found to be the case, although even a 1,000-fold excess of glutamine resulted in only a partial inhibition of growth, whereas the growth of HU469 was completely inhibited by only a 5-fold excess of glutamine. It is not clear from the present results why glutamine is such a weak inhibitor of the growth of HU471 and HU470 on methionine sulfoxide.

The transport results also showed that methionine completely inhibited that fraction of methionine sulfoxide transport brought about by the *metP*$^+$ system; this was most clearly seen in HU470 (*metE205 metP*$^+$ *glnP252*). However, previous results indicated that methionine sulfoxide is only a weak inhibitor of methionine transport (1). Indeed, this observation was used to argue that methionine sulfoxide is not transported by the *metP* system (1). The present work showed that this lack of inhibition could not be used as evidence for lack of transport of methionine sulfoxide by the *metP*$^+$ system. The lack of inhibition could be explained if there were two components to the *metP*$^+$ system, which worked in parallel and passed the substrate on to a third component. This suggestion was made previously for the *metD*$^+$ high-affinity methionine transport system in *E. coli* to account for the fact that, although D-methionine is a very weak inhibitor of L-methionine uptake, both isomers are transported by the *metD* system (13). To explain the present observations in *S. typhimurium*, one of the components working in parallel would recognize only L-methionine, whereas the other component would recognize L-methionine, D-methionine, L-methionine-DL-sulfoxide, and L-glutamine. The weak inhibition could also be explained if there were large differences in K_m between methionine and methionine sulfoxide. Further work is required to distinguish between these two possibilities.

Although it is now clear how methionine sulfoxide is transported into *S. typhimurium*, there are no reports of mutants lacking the ability to reduce either free methionine sulfoxide or methionine sulfoxide in proteins to methionine. A similar enzyme which reduces biotin sulfoxide has been described in *E. coli* (7). Mutants lacking the specific reductase carried mutations in four genes, suggesting that the enzyme is rather complex. Interestingly, these mutants are unimpaired in their ability to use methionine sulfoxide as a methionine source (7). It would be of interest to see whether the reductase acting on free methionine sulfoxide is also a complex enzyme and to determine its biological role.

ACKNOWLEDGMENTS

I thank Linda Kirk for excellent technical assistance and Andrew Cottam, Ken Sanderson, and Derek Smith for the strains.

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