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

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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 [14C]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: thiorodoxin, thiorodoxin 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 \(K_a\), about 0.1 \(\mu M\)) 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 sulfoxi-
mine (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 ap-
peared that methionine sulfoximine is trans-
ported by two different systems, and both sys-
tems had to be blocked to bring about full res-
istance 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 un-
able to grow. Therefore, it was concluded that
methionine sulfoximine, 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 (HU506),
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 B12 (cyanocobalamin) (17).

Media. Nutrient agar (code CM3) and nutrient
broth (code CM1) were supplied by Oxoid Ltd. Min-
imal medium contained the following (in grams per
liter): K2HPO4, 10.5; KH2PO4, 4.5; trisodium citrate·
2H2O, 0.47; (NH4)2SO4, 1; MgSO4·7H2O, 0.05; and
d-glucose, 4. Minimal agar was minimal medium con-
taining 0.5%, rather than 0.4%, glucose, solidified with
1.5% New Zealand agar (British Drug Houses, Ltd.).
Arabinose and galactose minimal agar, for the selec-
tion of Ara+ and Gal+ phenotypes in transduction
crosses, contained 1% L-arabinose or 1% β-galactose,
respectively, and lacked glucose and sodium citrate.
L-Methionine, D-methionine, L-methionine sulfo-
ne (Sigma Chemical Co.), and L-methionine-DL-
sulfoxide (Koch-Light Laboratories, Ltd.) were added to media
at 20 μg ml⁻¹. Solutions of L-glutamine (Sigma Chemi-
ical Co.) were freshly prepared and added to media
at 100 μg ml⁻¹. Vitamin B12 (British Drug Houses,
Ltd.) was added at 0.1 μg ml⁻¹.

Chemicals. L-[methyl-¹⁴C]methionine (60.2 mCi
mmol⁻¹; 2.23 GBq mmol⁻¹) was obtained from the
Radiochemical Centre. L-[¹⁴C]methionine-DL-
sulfox-
ide was prepared by incubating 0.42 mM L-[methyl-
¹⁴C]methionine for 2 h at room temperature with 0.3
or 1.6% (wt/vol) hydrogen peroxide in 1 ml of phos-
phate 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 chromato-
graphy plate, and the plate was run in tert-butyl alco-
hol-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 [¹⁴C]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 [¹⁴C]methionine sulfoxide was de-
termined by thin-layer chromatography in tert-butyl al-
cohol-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-methio-
nine sulfoxide 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 mini-
mal agar plus the indicated supplements. For experi-
ments with metE strains in supplemented minimal
medium, bacteria were grown overnight in 10 ml of
minimal medium plus L-methionine at 20 μg 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 ex-
perimental flasks. For the transport assays involving
metE strains, bacteria were grown in 50 ml of minimal
medium plus vitamin B12 at 0.1 μg ml⁻¹. Vitamin B12
was used, since, unlike L-methionine, it does not result in
the repression of the metP⁺ transport system (2; un-
published data). After centrifugation, the cultures
were washed with a culture volume of minimal med-
ium plus chloramphenicol at 200 μg ml⁻¹ and resus-
pended 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 per-
formed essentially as previously described (2). [¹⁴C]
methionine sulfoxide with or without unlabeled L-met-
hothione or L-glutamine was incubated for at least
2 min at 25°C. The assay was initiated by the addi-
tion 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 scintil-
lation 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 10⁶ phage to the donor bac-
teria in soft nutrient agar layers, and transductions
were performed directly on the minimal agar sur-
face (10); 0.05 ml of a donor phage lysate and 0.05 ml of an
overnight nutrient broth culture of the recipient bac-
teria 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
lysates (10^9 phage) were then added to 50-ml log-phase cultures of the same recipients, and the phage were propagated (16). The resulting titers were between 6 × 10^9 and 10 × 10^9 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 *met P* and *gln P* 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 B₁₂, as expected. All of the *met P*-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 *met E* *met P* *gln P* strains failed to grow on this compound; only HU435 and HU438 gave positive results. Of the six strains containing only the *met P* 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Origin or reference</th>
</tr>
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<tbody>
<tr>
<td>HU103</td>
<td>LT2 wild type</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>HU36</td>
<td>metE205 ara-9</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>HU439</td>
<td>metE205</td>
<td>HU36* transduction with HU103 as donor</td>
</tr>
<tr>
<td>HU425</td>
<td>metE205 metP760</td>
<td>HU163 transduction with HU103 as donor</td>
</tr>
<tr>
<td>HU478a</td>
<td>metE205 metP761 glnP251</td>
<td>HU426 transduction with HU103 as donor</td>
</tr>
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<td>HU479a</td>
<td>metE205 metP762 glnP253</td>
<td>HU427 transduction with HU103 as donor</td>
</tr>
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<td>metE205 metP763</td>
<td>HU181* transduction with HU103 as donor</td>
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<tr>
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<td>metE205 metP764</td>
<td>HU182* transduction with HU103 as donor</td>
</tr>
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<td>HU430a</td>
<td>metE205 metP765</td>
<td>HU108* transduction with HU103 as donor</td>
</tr>
<tr>
<td>HU431</td>
<td>metE205 metP766</td>
<td>HU109* transduction with HU103 as donor</td>
</tr>
<tr>
<td>HU468</td>
<td>metE205 metP767 glnP252</td>
<td>HU262 transduction with HU103 as donor</td>
</tr>
<tr>
<td>HU469a</td>
<td>metE205 metP768 glnP254</td>
<td>HU142 conjugation with HU36 as recipient</td>
</tr>
<tr>
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<td>metE205 metP1707</td>
<td>HU253* transduction with HU103 as donor</td>
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<td>HU434</td>
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<td>metE205 metP1712 glnP260</td>
<td>HU414* transduction with HU103 as donor</td>
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<td>metE205 metP763 ara-9*</td>
<td>HU84 transduction with HU103 as donor</td>
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<td>HU484</td>
<td>metE205 metP761 glnP251 ara-9* galK50*</td>
<td>HU151 mutagenesis with NG&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>K. E. Sanderson</td>
</tr>
<tr>
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<td>HU15 mutagenesis with NG&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>HU262</td>
<td>metE205 metP767 glnP252 galK50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>HU262 transduction with HU36 as donor</td>
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<tr>
<td>HU142</td>
<td>HfrK2 hisD23 metE205 metP768 glnP254</td>
<td>HU18 spontaneous MS&lt;sup&gt;+&lt;/sup&gt; mutant&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>HU18</td>
<td>HfrK2 hisD23</td>
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</tr>
<tr>
<td>HU424</td>
<td>metE205 metP767 glnP252 ara-9 galK50</td>
<td>HU423 transduction with HU36 as donor</td>
</tr>
<tr>
<td>HU423</td>
<td>metE205 metP767 glnP252 leu galK50</td>
<td>HU423 transduction with HU36 as donor</td>
</tr>
<tr>
<td>HU429</td>
<td>metE205 metP767 glnP252 ara-9 galK50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>HU423 transduction with HU36 as donor</td>
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<td>metE205 metP1709 glnP257</td>
<td>HU468 transduction with HU36 as donor</td>
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<td>metE205 metP1709 glnP258</td>
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<td>metE205 metP1712 glnP260</td>
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<tr>
<td>HU471</td>
<td>metE205 metP1712 glnP260</td>
<td>HU468 transduction with HU36 as donor</td>
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</tbody>
</table>

<sup>a</sup> These strains are all ara-9 and are fully described in reference 2.

<sup>b</sup> HU478, HU479, and HU430 were constructed by A. Cottam.

<sup>c</sup> These mutations were not listed in the genotypes given in reference 2.

<sup>d</sup> NG, N-Methyl-N'-nitro-N-nitrosoguanidine.

<sup>e</sup> MS*, Resistance to L-methionine-DL-sulfoximine at 50 µg ml⁻¹.
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

![Graph](image.png)

**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>
<thead>
<tr>
<th>Strain</th>
<th>General genotype</th>
<th>d-Methionine (20 µg ml$^{-1}$)</th>
<th>L-Methionine sulfoxide (20 µg ml$^{-1}$)</th>
<th>L-Methionine sulfoxide + L-glutamine (100 µg ml$^{-1}$)</th>
<th>Vitamin $B_{12}$ (0.1 µg ml$^{-1}$)</th>
<th>L-Methionine (20 µg ml$^{-1}$)</th>
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<tbody>
<tr>
<td>HU439</td>
<td>$metE205$ $metP^+$ $glnP^+$</td>
<td>+</td>
<td>+</td>
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<tr>
<td>HU425, HU428, HU429, HU431, HU433</td>
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<td>-</td>
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<td>-</td>
<td>+</td>
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<td>HU430</td>
<td>$metE205$ $metP$ $glnP^+$</td>
<td>-</td>
<td>±</td>
<td>-</td>
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<td>±</td>
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<td>HU435, HU438</td>
<td>$metE205$ $metP$ $glnP$</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Saline suspensions were streaked onto various media, and the results were scored after 24 h. +, Full growth response; ±, intermediate growth response; −, no growth.
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-[¹⁴C]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 Kₘ of methionine

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**Table 3. Growth of metE metP glnP recombinants on methionine sulfoxide**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Growth on minimal agar plus:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D-Methionine</td>
<td>L-Methionine sulfoxide</td>
<td>L-Methionine sulfoxide + L-glutamine</td>
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<tr>
<td>HU471</td>
<td>metE205 metP+ glnP+</td>
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<tr>
<td>HU470</td>
<td>metE205 metP+ glnP252</td>
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<td>HU469</td>
<td>metE205 metP767 glnP+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>HU468</td>
<td>metE205 metP767 glnP252</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Details as in Table 2.*
TABLE 4. Transduction of metE205 metP767 glnP252 ara-9 (HU424) on methionine sulfoxide

| Strain | Donor phage genotype | No. of transductants on: | % of transductants on methionine sulfoxide which were:
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Arabinose + vitamin B12</td>
<td>Methionine sulfoxide</td>
</tr>
<tr>
<td>HU471</td>
<td>metE205 metP* glnP*</td>
<td>180</td>
<td>189</td>
</tr>
<tr>
<td>HU470</td>
<td>metE205 metP* glnP252</td>
<td>246</td>
<td>147</td>
</tr>
<tr>
<td>HU469</td>
<td>metE205 metP767 glnP*</td>
<td>111</td>
<td>7</td>
</tr>
<tr>
<td>HU468</td>
<td>metE205 metP767 glnP252</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

A total of 0.5 ml of phage (10^9 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.

Phage were prepared by two cycles of lysis on the donor strains.

Minimal agar plus L-methionine-DL-sulfoxide.

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Uptake*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No addition</td>
</tr>
<tr>
<td>HU471</td>
<td>metE205 metP* glnP*</td>
<td>100</td>
</tr>
<tr>
<td>HU470</td>
<td>metE205 metP* glnP252</td>
<td>82.9</td>
</tr>
<tr>
<td>HU469</td>
<td>metE205 metP767 glnP*</td>
<td>3.9</td>
</tr>
<tr>
<td>HU468</td>
<td>metE205 metP767 glnP252</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Bacteria were grown in minimal medium plus vitamin B12.

Uptake of 0.70 μM L-[14C]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]).

70 μM L-methionine or 70 μM L-glutamine included in the assay.

700 μM L-glutamine.

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 15 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 \textit{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 \textit{d}-methionine transport by the \textit{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 \textit{metP}$^+$ system; this was most clearly seen in HU470 (\textit{metE205 metP}$^+$ glpP252). 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 \textit{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 \textit{metP}$^+$ system. The lack of inhibition could be explained if there were two components to the \textit{metP} system, which worked in parallel and passed the substrate on to a third component. This suggestion was made previously for the \textit{metD}$^+$ high-affinity methionine transport system in \textit{E. coli} to account for the fact that, although \textit{d}-methionine is a very weak inhibitor of \textit{L}-methionine uptake, both isomers are transported by the \textit{metD} system (13). To explain the present observations in \textit{S. typhimurium}, one of the components working in parallel would recognize only \textit{L}-methionine, whereas the other component would recognize \textit{L}-methionine, \textit{D}-methionine, \textit{L}-methionine-$\text{DL}$-sulfoxide, and \textit{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 \textit{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 \textit{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.

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LITERATURE CITED


