JOURNAL OF BACTERIOLOGY, June 1975, p. 1208-1215
Vol. 122, No. 3

Multiplicity of Oligopeptide Transport Systems in *Escherichia coli*

FRED NAIDER AND JEFFREY M. BECKER*

*Department of Pure and Applied Sciences, Richmond College, City University of New York, Staten Island, New York 10301 and Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37916**

Received for publication 10 March 1975

The ability of *Escherichia coli* K-12 4212 to utilize a variety of oligopeptides as sources of required amino acids was examined. Triornithine-resistant mutants of this strain were oligopeptide permease deficient (Opp$^-$) as judged by their inability to utilize $(Lys)_3$ and $(Lys)_4$ as sources of lysine and their resistance to the toxic tripeptide $(Val)_3$. These same mutants were able to grow when Met-Met-Met, Met-Gly-Met, Met-Gly-Gly, Gly-Met-Gly, Gly-Gly-Met, Gly-Met-Met, Met-Met-Gly, or Leu-Leu-Leu were supplied in place of the requisite amino acid. The system mediating the uptake of these peptides, herein designated Opr I, was not able to transport *N*-α-acetylated peptides nor the tetrapeptides Met-Gly-Met-Met, Met-Met-Gly-Met, or Met-Met-Met-Gly. Competition experiments indicated that trimethionine and trileucine enter *E. coli* K-12 via either Opp or Opr I. Analogous results were found using the methionine, leucine-requiring auxotroph *E. coli* B163. It appears that more than one oligopeptide transport system exists in *E. coli* and that the system mediating peptide uptake is complex.

The last 10 years have seen significant attention given to the structural specificity of the oligopeptide transport system of *Escherichia coli*. Studies carried out primarily in the laboratories of Gilvarg (6, 9, 16) and Payne (13, 15) have delineated many of the molecular features required for the recognition of peptides by a transport system designated as the oligopeptide permease (Opp) (1). By using competition experiments, whereby one peptide impedes entry of another peptide, and a mutation to triornithine resistance, which renders the microorganism unable to transport various peptides, it has been shown that a wide variety of homo-oligopeptides and mixed oligopeptides utilize Opp to enter *E. coli*. It is a currently held opinion that most oligopeptides, regardless of their amino acid composition or sequence enter *E. coli* via this common transport system (16).

In studies concerned with the stereospecificity of peptide transport in *E. coli* K-12 and *E. coli* B we observed that methionine-containing peptides are readily utilized by these bacteria (3). The structural requirements of the transport system mediating the uptake of these peptides by *E. coli* K-12 were similar in many respects to those reported for the Opp system. Thus, *N*-acetylated peptides were not transported, tripeptide methyl esters were utilized, and a size limit to transport was a chain length of five methionine residues.

In this report we present evidence that some peptides, specifically those containing methionine and leucine residues, may not enter *E. coli* exclusively via the Opp transport system. We delineate some structural requirements of the oligopeptide transport system mediating uptake of these peptides and designate it tentatively as Opr I. Finally, we propose that, in addition to the general oligopeptide transport system (Opp), there may exist systems, such as Opr I, which have more restricted side-chain specificity.

MATERIALS AND METHODS

Bacterial strains. *E. coli* B163 originally from the culture collection of Martin Lubin (8) was obtained from Z. Barak, Department of Biophysics, Weizmann Institute, Rehovot, Israel. The culture was received frozen as a suspension in 20% glycerol. The suspension was streaked onto a petri plate containing 1% yeast extract, 2% peptone, 2% dextrose, and 2% agar, and reisolated from a single colony. The phenotype of the reisolate corresponded to the markers *met*$^-$, *leu*$^-$, and *his*$^-$originally reported for strain B163. *E. coli* K-12 was received from B. Bachman of Yale University as strain CGSC 4212. The relevant genotype and distinguishing characteristics of this strain and the other strains used in this paper are represented in Table 1. Strain B163Tor, strain 4212 TorBN1, and 4212 TorBN2 were spontaneous mutants isolated from their respective parental strains after growth for 24 h in complete minimal medium supplemented with

TABLE 1. *List of E. coli strains*

Strain	Relevant genotype and distinguishing characteristics	Source
4212	*thi*-1, *metE70*, *lysA23*, *trypE38*, *purE42*, *proC32*, and *leu-6*	B. Bachman
4212 TorBN1	Same as 4212 except resistant to toxicity of triornithine	Derived from 4212 (see Materials and Methods)
4212 TorBN2	Same as 4212 TorBN1 except independent isolation	Derived from 4212 (see Materials and Methods)
B163	*met*, *leu*, *his*	Z. Barak
B163Tor	Same as B163 except resistant to toxicity of triornithine	Derived from B163 (see Materials and Methods)

triornithine (100 μg/ml), as described by Barak and Gilvarg (1). Each of the derivative strains were streaked twice to reisolate individual colonies, and tested for desired distinguishing characteristics, e.g., triornithine resistance.

Media and growth conditions. For growth studies the synthetic Vogel-Bonner mineral medium E (17) was used. Other minimal media such as Davis-Mingioli (5) were used occasionally. The growth response to various peptides was identical for all the minimal media tested. The amino acid or peptide under test was sterilized by filtration through membrane filters and added aseptically to 5 ml of minimal medium in a Klett tube. Sterile glucose (20% [wt/vol]) solution was added aseptically to bring the growth medium to a final concentration of 0.2% (wt/vol) in glucose. The inoculum consisted of 0.1 ml of an overnight culture that had been grown at 37 C in minimal medium supplemented with glucose (0.2% [wt/vol]) and the appropriate growth factors. For strains B163 and B163Tor, methionine (10 μg/ml), leucine (10 μg/ml), and histidine (10 μg/ml) were added to minimal medium. The necessary growth factors added to minimal medium for strains 4212, 4212 TorBN1, and 4212 TorBN2 included methionine (10 μg/ml), thiamine (0.1 μg/ml), lysine (10 μg/ml), tryptophan (10 μg/ml), adenine (10 μg/ml), proline (10 μg/ml), and leucine (10 μg/ml). The cultures were incubated at 37 C with shaking. Turbidity was measured in a Klett-Summerson colorimeter fitted with a blue filter (400 to 420 nm).

Chemicals. Most of the methionine-containing peptides were purchased from Schwarz-Mann Co., Orangeburg, N. J. Trileucine was purchased from Sigma Chemical Co., St. Louis, Mo. The synthesis of noncommerical peptides including pentamethionine, the acetylated methionine peptides, and the methyl ester derivatives were reported in the literature (11). Dilysine, trilysine, and tetralysine were purchased from Miles-Yeda, Rehovot, Israel. Triornithine and trivaline were received as a generous gift from Z. Barak. All other chemicals were reagent grade or the purest commercially available.

Cell extracts. The *E. coli* strains were grown in a 2-liter Erlenmeyer flask in 500 ml of minimal medium supplemented with glucose and the necessary growth factors. The cultures were incubated at 37 C without shaking. Cells were harvested by centrifugation (2,000 × *g*, 15 min) in the late exponential stage (80 Klett units), washed twice with sterile, 0.9% NaCl at 4 C, and resuspended in 20 ml of sterile 0.025 M phosphate buffer, pH 7.2. The cell suspension, along with 40 g of glass beads (50 μm, Minnesota Mining and Manufacturing Co.), was added to the 50-ml Omnimixer stainless-steel cup (Ivan Sorvall Co.) and homogenized at top speed for 10 min. The beads were removed by filtration through a sintered-glass funnel. Unbroken cells and debris were removed by centrifugation (15,000 × *g*, 15 min) and the supernatant cell extract was stored at −20 C. The protein content as measured by the Lowry method (10), with bovine serum albumin as standard, was 350 μg of protein per ml of cell extract.

Peptidase assay and electrophoresis. A portion of cell extract (usually 100 μl) was incubated at 30 C with an equal volume of various peptide solutions (2 mg/ml in distilled water). At various times, samples of the reaction mixture were removed and subjected to paper electrophoresis in a model LT-36 electrophoresis tank (Savant Instruments). Pyridine acetate buffer (pH 3.5) was prepared from glacial acetic acid-pyridine-water (10:1:89, by volume). Samples were applied to Whatman 3 MM paper and subjected to a gradient of 5 V/cm for 1 to 2 h. After electrophoresis the paper was dried, dipped in a solution of ninhydrin (0.5% [wt/vol] in 95% aqueous acetone), and developed by heating in an oven.

RESULTS

Growth of E. coli K-12 strains in the presence of triornithine. A triornithine-resistant mutant of *E. coli* 4212 was isolated using the procedure described. The growth response of this mutant and the parent strain in the presence of lysine, $(Orn)_3$, $(Lys)_3$, and $(Lys)_4$ are represented in Fig. 1. The Tor mutant is insensitive to the presence of $(Orn)_3$ but fails to grow when $(Lys)_3$ or $(Lys)_4$ are the nutritional supplement for lysine. In contrast, the parent strain is completely inhibited by the presence of $(Orn)_3$,

but can utilize either $(Lys)_3$ or $(Lys)_4$, as can other *E. coli* strains reported in the literature (16). Peptidases contained in cell extracts of *E. coli* 4212 and *E. coli* 4212 TorBN1 hydrolized both $(Lys)_3$ and $(Lys)_4$ at approximately equal rates as judged using paper electrophoresis (J. M. Becker and F. Naider, unpublished data).

Characterization of E. coli 4212 TorBN1. The inability of *E. coli* 4212 TorBN1 to utilize $(Lys)_3$ and $(Lys)_4$ was presumptive evidence that this mutant was Opp$^-$. We investigated this possibility further using other peptides believed to enter *E. coli* by the Opp system. Strains of *E. coli* K-12 are known to be sensitive to valine (7). As seen in Fig. 2, *E. coli* 4212 is completely inhibited by trivaline in the growth medium. The analogous Tor mutant, however, is not affected by this toxic tripeptide. In addition, strain 4212 TorBN1 grew on a number of dipeptides containing methionine or lysine residues but could not utilize acetylated tripeptides, as judged by its failure to grow on Ac-Met-Met-Met and Ac-Met-Gly-Met. These growth responses are similar to those reported for other Tor mutants of *E. coli* (16).

Growth of E. Coli 4212 TorBN1 on tripeptides and tetrapeptides containing methionine. The growth of *E. coli* 4212 and the corresponding Tor mutant were measured in response to various methionine-containing peptides. The growth medium was supplemented with peptides so that the Met residue concentration was 6.7×10^{-5} M in all cases. The data represented in Fig. 3 and Table 2 illustrate the following findings: (i) all methionine-containing tripeptides tested are utilized by both the parent strain and Tor mutant. Growth was in general equivalent to that on free methionine except in the case of Gly-Met-Gly, which was a poor growth substrate for the Tor mutant; (ii) trileucine can serve as a nutritional source for leucine in *E. coli* 4212 and *E. coli* 4212 TorBN1; (iii) the methionine-containing tetrapeptides are not utilized by the Tor mutants although they are utilized by the corresponding $(Orn)_3$-sensitive parental strains. (iv) Met-Met-Met-OMe serves as a growth substrate for all strains tested. The lack of growth on the nonutilized peptides was not due to the lack of peptidases as judged, for example, by the breakdown of Met-Gly-Met-Met to methionine after incubation with a cell extract (Fig. 4). An additional

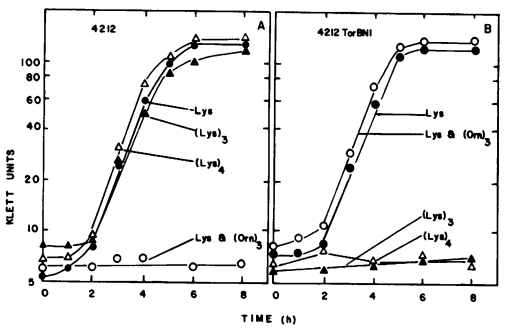

FIG. 1. *Growth of E. coli strains in the presence of various peptides. Minimal medium included all required nutrients except lysine. Symbols: A represents growth of E. coli 4212 on minimal media supplemented with* L-*lysine or* $(Lys)_2$ (●); L-*lysine plus triornithine (100 μg/ml)* (○); $(Lys)_3$ (▲); *and* $(Lys)_4$ (△). *B shows growth of E. coli 4212 TorBN1 on the minimal medium with the same supplementation. The* L-*lysine residue concentration in each growth tube was* 6.7×10^{-5} *M.*

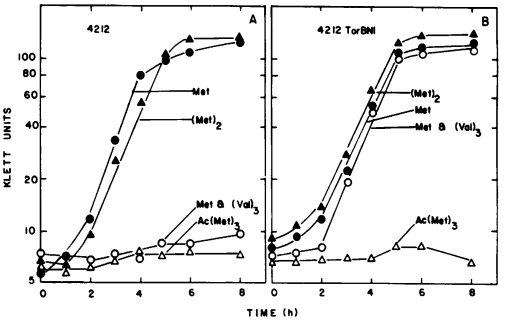

FIG. 2. *Growth of E. coli strains in the presence of various peptides. Minimal media included all required nutrients except methionine. A shows growth of E. coli 4212 on minimal media supplemented with* L-*methionine* (●); L-*methionine plus trivaline (100 μg/ml)* (○); $(Met)_2$ (▲); *and Ac-*$(Met)_3$ (△). *B represents growth of E. coli 4212 TorBN1 on minimal media with the same supplementation. The* L-*methionine residue concentration in each growth tube was* 6.7×10^{-5} *M.*

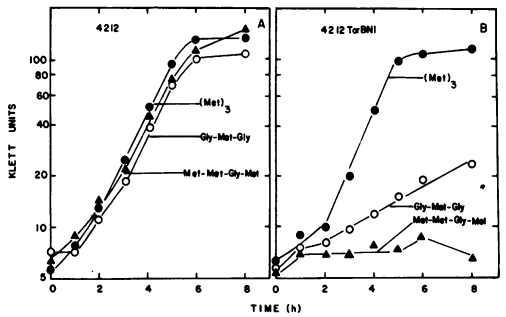

FIG. 3. *Growth response of E. coli strains on methionine-containing oligopeptides. The minimal media was complete except for the omission of methionine which was supplied in the form of* $(Met)_3$ (●); *Gly-Met-Gly* (○); *and Met-Met-Gly-Met* (▲). *A shows growth of E. coli 4212 and B represents the response of E. coli 4212 TorBN1.*

TABLE 2. *Growth response of various E. coli strains to peptides*

Peptide[a]	Growth response of *E. coli* strain:			
	4212	4212 TorBN1	B163	B163Tor
Lys-lys	+[b]	+	ND[c]	ND
Met-Met, Gly-Met, Met-Gly	+	+	+	+
Met-Met-Met	+	+	+	+
Ac-Met-Met-Met	0[d]	0	0	0
Ac-Met-Gly-Met	0	0	0	0
Met-Met-Met-OMe	+	+	+	+
Gly-Gly-Met	+	+	+	+
Met-Gly-Gly	+	+	+	+
Gly-Met-Met	+	+	+	+
Met-Met-Gly	+	+	+	+
Met-Gly-Met	+	+	+	+
Gly-Met-Gly	+	+/−[e]	+	+/−
Met-Gly-Met-Met	+	0	+	0
Met-Met-Gly-Met	+	0	+	0
Met-Met-Met-Gly	+	0	+	0
Met-Met-Gly-Gly	ND	ND	+	0
Met-Met-Met-Met-Met	0	0	0	0
Lys-Lys-Lys	+	0	ND	ND
Lys-Lys-Lys-Lys	+	0	ND	ND
Leu-Leu-Leu	+	+	+	+
Orn-Orn-Orn[f]	0	+	+/−	+

[a] All peptides contain only amino acids in the L-configuration.
[b] +, Growth equivalent to that on L-methionine.
[c] ND, Not determined.
[d] 0, No growth in 8 h.
[e] +/−, Generation time at least twice that on L-methionine.
[f] Growth measured in a complete media supplemented with all free amino acids required plus triornithine (100 μg/ml).

Tor mutant of *E. coli* 4212 was isolated independently from a single colony. This Tor mutant, strain 4212 TorBN2, gave growth responses similar to those represented in Fig. 1 through 3 and Table 2.

Competition experiments leading to growth inhibition. Evidence that oligopeptides utilize a common transport system has been obtained using experiments in which different peptides competed with one another for entry into the cell (12). We therefore carried out a series of experiments whereby the ability of trileucine and trimethionine to compete with each other and a number of peptides known to use the Opp system was determined. Cells used as an inoculum in competition experiments were grown overnight in a suboptimal amount (5 μg/ml) of methionine or leucine. The experimental medium was supplemented with two peptides, a growth factor-containing peptide, and the peptide competitor under examination. The results of these experiments are summarized in Tables 3 and 4. The data show that trilysine and tetralysine do not compete with $(Leu)_3$ or $(Met)_3$ for entry into *E. coli* 4212 when present at equimolar concentrations and that $(Lys)_4$ even at 10 times the concentration of trileucine or trimethionine is only slightly inhibitory. In contrast competition studies carried out in *E. coli* 4212 show that $(Met)_3$ effectively competes with $(Leu)_3$, $(Lys)_3$, and $(Lys)_4$ for entry into the bacterium (Table 3).

Competition studies carried out in *E. coli* 4212 TorBN1 show that $(Lys)_4$ at 10-fold molar excess does not compete with trimethionine for entry into these cells (Table 4). Moreover, whereas $(Met)_3$ was an effective competitor with $(Leu)_3$ in the parent strain it completely prevents its entry into the Tor mutant when present at equimolar concentrations. In contrast, equimolar concentrations of trileucine do not affect the growth of 4212 TorBN1 on trimethionine and a 10-fold molar excess of trileucine competes only slightly.

Competition leading to reversal of triornithine toxicity. Triornithine is toxic to strain 4212. Any oligopeptide that can prevent this peptide from entering the cell should be able to overcome the inhibitory effect of $(Orn)_3$. Competition in this system should lead, therefore, to an enhanced growth response. Trimethionine at one-tenth the molar concentration is

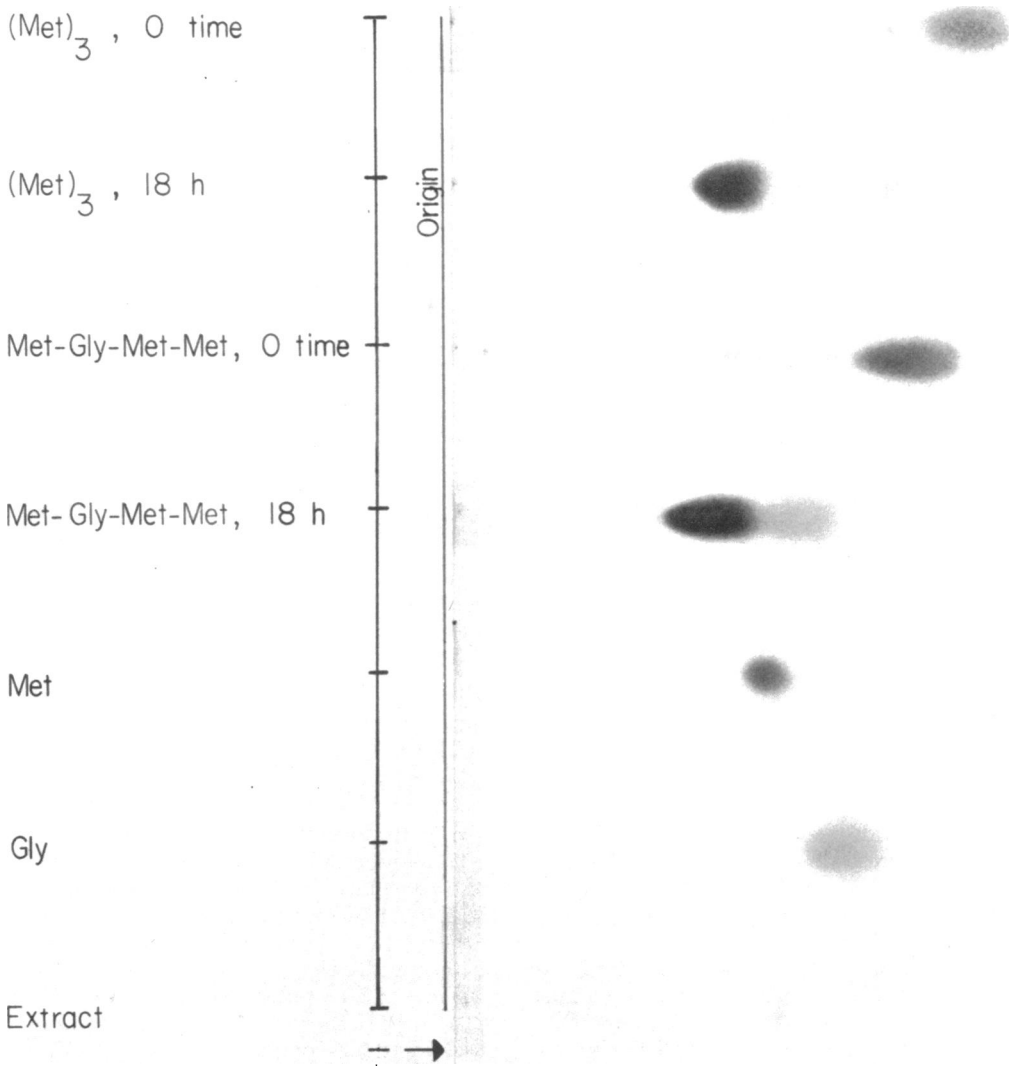

FIG. 4. *Paper electrophoresis at pH 3.5 of a 18-h incubation mixture of Met-Met-Met or Met-Gly-Met-Met and a cell extract from 4212 TorBN1. Markers are represented by Met and Gly.*

effective in overcoming triornithine toxicity (Fig. 5). The reversal of growth inhibition is complete when the molar ratio of trimethionine to triornithine is approximately 1:1.

Growth of E. coli B163 on various peptides. An analogous series of growth experiments was conducted with a strain of *E. coli* B auxotrophic for methionine and leucine. This strain proved to be only mildly sensitive to triornithine, exhibiting a slight growth inhibition when grown in the presence of this peptide. We were still able to isolate, however, a mutant of *E. coli* B163 that was totally resistant to $(Orn)_3$ (see Materials and Methods). The growth response of *E. coli* B163 and *E. coli* B163Tor to a variety of di-, tri-, and tetrapeptides is summarized in Table 2. The growth results obtained with *E. coli* B were completely consistent with those found with *E. coli* K-12.

DISCUSSION

In this paper we have shown that Leu-Leu-Leu, Met-Met-Met as well as six tripeptides containing methionine and glycine satisfy the nutritional requirements of Tor mutants of *E.*

TABLE 3. *Peptide competition in E. coli 4212*

Growth peptide	Competing peptide[a]			
	$(Met)_3$	$(Leu)_3$	$(Lys)_3$	$(Lys)_4$
$(Met)_3$		– ++ (10X)	–	– +(10X)
$(Leu)_3$	++ +++(10X)[b]			– +(10X)
$(Lys)_3$	+++			
$(Lys)_4$	+ +++(10X)	+ +++(10X)		

[a] +++, complete inhibition for 8 h; ++, one-third maximal growth for 8 h; +, two-thirds maximal growth for 8 h; –, no effect (or maximal growth attained). The competing peptide and the growth peptide were supplemented to the growth medium at approximately equimolar concentration.

[b] 10X is competitor present in 10-fold molar excess.

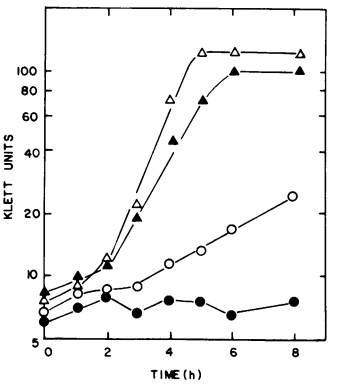

FIG. 5. *Reversal of triornithine toxicity by trimethionine in E. coli 4212. The growth of strain 4212 in complete minimal media supplemented with* $(Orn)_3$ *at 100 μg/ml (27.7 × 10⁻⁵ M) was measured in the presence of no* $(Met)_3$ (●); $(Met)_3$ *at 2.2 × 10⁻⁵ M* (○); *and* $(Met)_3$ *at 22.0 × 10⁻⁵ M* (▲). *The control tube contained minimal medium supplemented with all the required growth factors and* $(Met)_3$ *at 2.2 × 10⁻⁵ M* (△).

TABLE 4. *Peptide competition in E. coli 4212 TorBN1*

Growth peptide	Competing peptide[a]			
	$(Met)_3$	$(Leu)_3$	$(Lys)_3$	$(Lys)_4$
$(Met)_3$		– +(10X)[b]	–	– –(10X)
$(Leu)_3$	+++			– –(10X)

[a] +++, Complete inhibition for 8 h; ++, one-third maximal growth for 8 h; +, two-thirds maximal growth for 8 h; –, no effect (or maximal growth attained). The competing peptide and the growth peptide were supplemented to the growth medium at approximately equimolar concentration.

[b] 10X is competitor present in 10-fold molar excess.

coli K-12 4212. These Tor mutants were judged to be Opp⁻ based on their loss of ability to utilize $(Lys)_3$, $(Lys)_4$ and the methionine-containing tetrapeptides examined. Furthermore, $(Val)_3$, another peptide believed to enter *E. coli* via the Opp system, was not toxic to the Tor mutant although it was highly toxic to the parental strain. Neither $(Lys)_3$ nor $(Lys)_4$, both peptides believed to be transported by the Opp transport system, effectively compete at a 10-fold molar excess with Met-Met-Met for entry into either the parent strain or the Tor mutant. Thus, using Opp⁻ mutants and competitive inhibition of entry we have demonstrated that some tripeptides can enter *E. coli* via a transport system or systems other than that described in detail by Gilvarg, Payne et al. (6, 9, 13, 15, 16). We wish to designate this new peptide transport system as Opr I, an oligopeptide transport system of restricted side-chain specificity. All previous Opp⁻ strains isolated by the same procedure reported in this paper and subjected to genetic analysis were mapped as deletion mutants (Z. Barak, personal communication). In addition, strains 4212 Tor BN1 and 4212 Tor BN2 did not revert to growth on trilysine after nitrosoguanidine mutagenesis. Thus, the triornithine-resistant strains used in this paper were deletion mutants or multisite mutants. It is reasonable, therefore, that Opr I is a distinct transport system rather than a form of the Opp system with modified affinity.

The structural and stereochemical requirements of the Opr I transport system are, at present, not completely defined. The following conclusions, however, can be surmised from the results of both growth and competition experiments. The transport system(s) which accepts the methionine-containing tripeptides examined also seem to accept trileucine since $(Met)_3$ competes with $(Leu)_3$ utilization in Opp⁻ strains. Peptides containing only lysine are not recognized by this system as judged by the lack of competition of these peptides with trimethionine entry and the negative growth response of *E. coli* 4212 Tor BN1 on trilysine or tetralysine. The Opr I system resembles the Opp system in its requirement for a free or positively charged amine terminus and its lack of requirement for a free carboxyl terminus. Thus, the transport system present in *E. coli*

4212 TorBN1 does not accept *N*-acetylated tripeptides but appears to recognize tripeptide methyl ester. The size limit of Opr I, however, appears different from Opp based on the observation that Tor mutants do not grow on tetrapeptides containing methionine, whereas the triornithine-sensitive parental strain utilizes these tetrapeptides. Assuming that the spontaneous Tor mutation does not alter greatly the cell envelope of the parental strain (1), this size restriction of the Opr I system cannot be due to the same sieving effect which has been used to explain the size limitation in the Opp system (16). Finally, we have found that D-Met-L-Met-L-Met can enter *E. coli* 4212 but is excluded by the corresponding Tor mutant (3). It appears, therefore, that the Opp and Opr I systems have different stereochemical requirements.

The possibility that a second route may exist for the entry of peptides in *E. coli* has been previously suggested by Payne (12, 13). Thus the entry of glycyl-glycyl-norleucine into *E. coli* W (M-26-26) in the presence of peptide competitors (13) and the growth of *E. coli* W (M-123 Tor) on Gly-Leu-Gly (12) were attributed to permeation of the cell by a mechanism other than the Opp system. Furthermore, since the structure of the dipeptide carnosine precluded its entry into *E. coli* W (M-123) via either the dipeptide or oligopeptide transport system, it was proposed that another peptide uptake system may exist (14). As a possible explanation it was suggested that one of the other routes might be a diffusive process whereby neutral peptides enter *E. coli* (13). We believe, however, that the results of the present study make simple diffusion an unlikely explanation for the growth of *E. coli* B163Tor and 4212 TorBN1 on trileucine and trimethionine. Rapid growth on both of these peptide substrates was obtained at 6.7×10^{-5} M in amino acid residue concentration. In addition, the uptake of these peptides exhibited the characteristics generally observed for carrier type transport systems, that is, structural and stereochemical specificity.

We should note that most of the work of Gilvarg, Payne and their co-workers was carried out using *E. coli* W (16). Recent genetic studies show that a relatively small population of *E. coli* strains exhibit up to a 23% difference in genome size (4). It is possible, therefore, that the Opr I system is present only in *E. coli* K-12 and may be missing in *E. coli* W. The great similarity of our results with *E. coli* B163, however, suggests that Opr I-like systems may exist in other strains as well. Further work with a variety of strains is necessary to determine the exact nature of oligopeptide transport systems in *E. coli*.

It is apparent from the competition data that trimethionine may enter these strains of *E. coli* via both the Opp and Opr I systems. Thus, whereas $(Met)_3$ enters via the Opr I system in the Tor mutants, it presumably has some affinity for the Opp system, as it competes with $(Lys)_4$ and $(Lys)_3$ for entry into *E. coli* 4212. Trimethionine also protects *E. coli* B163 and *E. coli* 4212 against the toxic effects of $(Orn)_3$. The competition experiments point again to the different affinities that various oligopeptides have for peptide transport systems (12). It is evident from the data that $(Met)_3$ competes more effectively with $(Lys)_3$, $(Lys)_4$, or $(Orn)_3$ than does $(Leu)_3$ at comparable concentrations. Furthermore $(Met)_3$ completely excludes $(Leu)_3$ from the Tor mutant when present at equimolar concentrations, whereas a 10-fold molar excess of $(Leu)_3$ only slightly inhibits the growth of the same Tor mutant on $(Met)_3$. The exact effect of $(Leu)_3$ on $(Met)_3$ utilization is clouded by the finding that $(Leu)_3$ may be somewhat toxic when present at high concentration (F. Naider and J. Becker, unpublished observations). All of these findings suggest that the peptide transport phenomenon may be more complex than previously realized.

It was suggested that the existence of one general transport system capable of accepting oligopeptides containing many different amino acid residues is consistent with the huge number of permutations in structure that could exist even at the tripeptide level (16). It does not seem plausible that separate transport systems could have evolved for each oligopeptide able to enter a microorganism. Yet it is not unreasonable that a few oligopeptide transport systems could exist with different affinities for various classes of oligopeptides. Such transport systems might have overlapping specificities. It is possible that the Opp system serves as a general transport system for peptides, and that other, more specific, system(s) such as Opr I also exist.

It has come to our attention that a Tor mutant of *E. coli* K-12 can take up trithreonine and grows exponentially on trileucine (2). Furthermore, it was possible to isolate a mutant of this bacterium that could no longer take up trithreonine and grew poorly on limiting concentrations of trileucine. These data are consistent with our proposal that an additional oligopeptide transport system(s) exists in *E. coli* K-12 and will be discussed in detail in the following paper (2).

ACKNOWLEDGMENTS

This investigation was supported by grants GB-40686 and GB-40629 from the National Science Foundation, a grant from the Brown-Hazen Program of the Research Corporation,

and grant 10132 from the Faculty Award Research Program of CUNY.

The excellent technical asistance of Susan Winslow is greatly appreciated. We wish to thank Zeev Barak and Stuart Riggsby for critical evaluation of the manuscript and helpful suggestions, many of which were incorporated into the text.

LITERATURE CITED

1. Barak, Z., and C. Gilvarg. 1974. Triornithine-resistant strains of *Escherichia coli:* isolation, definition, and genetic studies. J. Biol. Chem. **249**:143-148.
2. Barak, Z., and C. Gilvarg. 1975. Specialized peptide transport system in *Escherichia coli*. J. Bacteriol. **122**:1200-1207.
3. Becker, J. M., and F. Naider. 1974. Stereospecificity of tripeptide utilization in a methionine auxotroph of *Escherichia coli* K-12. J. Bacteriol. **120**:191-196.
4. Brenner, D. J., G. R. Fanning, F. J. Skerman, and S. Falkow. 1972. Polynucleotide sequence divergence among strains of *Escherichia coli* and closely related organisms. J. Bacteriol. **109**:953-965.
5. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17-28.
6. Gilvarg, C., and E. Katchalski. 1965. Peptide utilization in *Escherichia coli*. J. Biol. Chem. **240**:3093-3098.
7. Glover, S. W. 1962. Valine resistant mutants of *Escherichia coli* K-12. Genet. Res. **3**:448-460.
8. Kessel, D., and M. Lubin. 1963. On the distinction between peptidase activity and peptide transport. Biochim. Biophys. Acta **71**:656-663.
9. Losick, R., and C. Gilvarg. 1966. Effect of α-acetylation on utilization of lysine oligopeptides in *Escherichia coli*. J. Biol. Chem. **241**:2340-2346.
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
11. Naider, F., J. M. Becker, and E. Katzir-Katchalski. 1974. Utilization of methionine-containing peptides and their derivatives by a methionine-requiring auxotroph of *Saccharomyces cerevisiae*. J. Biol. Chem. **249**:9-20.
12. Payne, J. W. 1968. Oligopeptide transport in *Escherichia coli:* specificity with respect to side chain and distinction from dipeptide transport. J. Biol. Chem. **243**:3395-3403.
13. Payne, J. W. 1971. The requirement for the protonated α-amino group for the transport of peptides in *Escherichia coli*. Biochem. J. **123**:245-253.
14. Payne, J. W. 1973. Peptide utilization in *Escherichia coli:* studies with peptides containing β-alanyl residues. Biochim. Biophys. Acta **298**:469-478.
15. Payne, J. W. 1974. Peptide transport in *Escherichia coli:* permease specificity towards terminal amino group substituents. J. Gen. Microbiol. **80**:269-276.
16. Payne, J. W., and C. Gilvarg. 1971. Peptide transport. Adv. Enzymol. **35**:187-244.
17. Vogel, H. J., and D. M. Bonner. 1965. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97-106.