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Specialized Peptide Transport System in *Escherichia coli*

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Trileucine is utilized as a source of leucine for growth of strains of *Escherichia coli* K-12 that are deficient in the oligopeptide transport system (Opp). Trithreonine is toxic to *E. coli* K-12. Opp$^-$ mutants of *E. coli* K-12 retain complete sensitivity to this tripeptide. Moreover, *E. coli* W, which is resistant to trithreonine, can utilize this tripeptide as a threonine source and this capability is fully maintained in *E. coli* W (Opp$^-$). A spontaneous trithreonine-resistant mutant of *E. coli* K-12 (Opp$^-$) has been isolated that has an impaired growth response to trileucine and is resistant to trithreonine. Trileucine competes with the uptake of trithreonine as measured by its ability to relieve trithreonine toxicity in *E. coli* K-12. It is concluded that trileucine as well as trithreonine are transported into *E. coli* K-12 or W by a common uptake system that is distinct from the Opp system. Trimethionine can act as a competitor of trileucine or trithreonine-supported growth and as an antagonist of trithreonine toxicity in Opp$^-$ mutants. It is concluded that trimethionine is recognized by the trileucine-trithreonine transport system. Trithreonine, trimethionine, and trileucine are also transported by the Opp system, as they all relieve triornithine toxicity towards *E. coli* W and compete with tetralysine utilization as lysine source for growth of a lysine auxotroph of this strain.

Amino acid-requiring mutants of *Escherichia coli* can grow on peptides containing the requisite amino acid. Evidence has been presented that this ability stems from the occurrence in *E. coli* of transport systems for peptides and an extensive intracellular array of peptidases (5, 12, 16-18). It has already been established that there are at least two distinct peptide transport systems. One system is limited to dipeptides, whereas the other is able to transport oligopeptides as well as dipeptides (8). This latter transport system has been designated as the oligopeptide permease (Opp).

It has been possible to select for spontaneous Opp$^-$ mutants of *E. coli* by exposing cultures of the organism to the toxic peptide triornithine. The triornithine-resistant (TOR) survivors from such a treatment appear to consist largely of mutants that have lost not only the capacity to take in triornithine, but many other oligopeptides as well (1, 3, 4, 8-11, 13, 15). Mutations responsible for the Opp$^-$ phenotype have been shown to lie at a single locus on the *E. coli* chromosome (1, 3). It has also been observed that oligopeptides containing different amino acids can compete with the transport of one another (8-12). The peptides that have been tested and shown to share the same oligopeptide transport system include some with basic amino acid residues (Lys, Orn, Arg), neutral residues (Gly, Ala, Leu, Val), aromatic residues (Tyr, Trp, Phe), hydroxy residues (Ser, Thr), and sulfur residues (Met). Even oligopeptides containing unusual amino acids such as norleucine, norvaline (9), sarcosine (9, 11), other *N*-alkyl amino acids (14), ϵ-acetylated lysine (7, 12), β-alanine (13), and homoserine phosphate (4) were found to utilize the oligopeptide transport system. These findings have served to define an Opp system that shows a very low degree of specificity for the side chains of the amino acid residues. This contrasts sharply with the stringency of other transport systems but can be rationalized as representing a satisfactory solution for the cell to the problem of how to cope with the enormous diversity of substrates inherent in having 20 different amino acids from which naturally occurring peptides are derived (5, 16).

It has been reported that Gly-Leu-Gly could be utilized by an Opp$^-$ strain (8). However, the high concentration of this tripeptide needed to support rapid growth and the nature of the amino acid residues, which would facilitate membrane solubility, made it likely that in this case the transport was diffusion mediated.

In the course of studying peptide transport in *E. coli* K-12 TD-V (1), we have observed additional examples of tripeptide utilization (Leu_3) and toxicity (Thr_3) in Opp$^-$ mutants that

indicate the existence of an additional peptide transport system. Evidence is also presented concerning the existence of this additional peptide transport system in strains of *E. coli* W.

MATERIALS AND METHODS

Chemicals. The peptides L-lysyl-L-*p*-fluorophenylalanine (Lys-*p*-F-Phe) and L-lysyl-L-lysyl-*p*-fluorophenylalanine (Lys_2-*p*-F-Phe) were synthesized by T. E. Fickel in this department. Di-L-lysine trihydrochloride, tri-L-lysine tetrahydrochloride (Lys_3), tetra-L-lysine pentahydrochloride (Lys_4), and tri-L-threonine monohydrate (Thr_3) were purchased from Fox Chemical Co. (Los Angeles, Calif.); the latter tripeptide was also purchased from Cyclo Chemical (Los Angeles, Calif.). Tri-L-leucine (Leu_3), di-L-valine, tri-L-valine (Val_3), and tri-glycine were also purchased from Cyclo Chemical tri-L-methionine (Met_3) from Mann Research Laboratories (New York), and tri-L-ornithine tetrahydrochloride (Orn_3), L-prolyl-L-phenylalanine and L-prolyl-L-phenylalanyl-L-lysine from Miles-Yeda (Rehovot, Israel). All the peptides were examined for purity by paper electrophoresis, and the amino acid composition was sometimes confirmed by amino acid analysis after hydrolysis. The purity of trithreonine was also tested by thin-layer chromatography by using the mixture ethanol-acetic acid-water (45:5:12.5) as solvent. Most of the peptides were free from impurities. Trithreonine, however, contained about 5% free threonine, trilysine, and trimethionine contained about 2% of the monomer.

Bacterial strains. Strains used in this investigation are listed in Table 1.

Media. The minimal medium used was medium VB (19). The required amino acids were added at a concentration of 50 μg/ml. A medium meeting all the amino acid requirements of a strain is referred to as a full medium. A full medium devoid of a certain amino acid is identified as VB minus the specific amino acid. Sometimes streptomycin (50 μg/ml) was added to the media which were then called VB full Sm, or VB minus amino acid Sm, respectively.

Bacterial growth. The rate of growth and the yield of bacteria were followed by measuring the optical density as a function of time in a Klett-Summerson colorimeter, using a 660-nm filter. The bacteria were grown in Klett tubes containing 5 ml of medium and were incubated with shaking (175 rpm) at 37 C. The peptides were added to the sterile medium aseptically. Tubes were inoculated with 1 to 2% (vol/vol) of an overnight culture.

Paper electrophoresis. Separation of neutral peptides was carried out on Whatman no. 1 paper with a potential gradient of 3,000 V for 2 h in 7% formic acid, pH 1.9. Charged peptides were subjected to electrophoresis for 30 to 60 min in 0.062 M pyridine-acetate buffer, pH 3.5. Peptide positions were determined using development with ninhydrin.

TABLE 1. *Bacterial strains.*

Strain	Genotype	Relevant phenotype	Origin	
			Parent	Reference
E. coli W		Wild type.		ATCC 9637
E. coli Bb		Wild type.		
TD-V	F⁻, λ⁻, *thr-1, leu-6, proA2, his-4, argE3, lac Y1, gal K2, ara-14, xyl-5, mtl-1, thi-1, sup E44, sup-37, str-31, tsx-33,* (*att φ80-tonB-trp*)^{del}	Requires threonine, leucine and proline	*E. coli* K-12 AB1157	1
TD-V-Tor-2, -3, -5	*opp*-I^{del} (otherwise like strain TD-V)[a]	Resistance to triornithine, trivaline, and lys_2-*p*-F-Phe. Inability to utilize Pro-Phe-Lys. Otherwise like strain TD-V.	TD-V	1
TD-V-TTR	*ttr*-I (otherwise like strain TD-V)	Resistance to trithreonine. Ability to utilize trithreonine. Otherwise like strain TD-V.	TD-V	see Results
TD-V-TOR-2-TTR	*opp*-II (otherwise like strain TD-V-TOR-2)	Resistance to trithreonine. Poor ability to utilize trileucine and trithreonine. Otherwise like strain TD-V-TOR-2.	TD-V-TOR-2	see Results
M-26-26	*lys A*	Requires lysine	*E. coli* W	ATCC 27798
TL3	*lys A, thr B*	Requires lysine and threonine	M-26-26	4
TL3-TOR	*opp*-I (otherwise like strain TL3)	Resistance to triornithine and Lys_2-*p*-F-Phe. Inability to utilize tri- and tetralysine. Otherwise like strain TL3.	TL3	4

[a] Deletion was determined by inability to revert under the influence of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (*In* L. A. Manson [ed.], *Biomembranes*, vol. 7, in press).

RESULTS

Utilization of trileucine by E. coli K-12 TD-V and its Opp⁻ mutants. *E. coli* K-12 TD-V-TOR-2 utilized trileucine (50 μg/ml) as a source of leucine for growth despite its oligopeptide transport deficiency. The growth of this Opp⁻ strain with trileucine (Fig. 1B) was similar to that observed under the same conditions with the Opp⁺ strain TD-V (Fig. 1A). Moreover, in both strains the tripeptide supported growth rates almost identical to that obtained with the monomer. These observations were confirmed with two other Opp⁻ strains of TD-V (TD-V-TOR-3 and TD-V-TOR-5). The rates of growth and the yields of bacteria were similar in both Opp⁺ and Opp⁻ strains even when using lower concentrations of trileucine (5, 10, and 20 μg/ml). These findings suggested the presence in *E. coli* K-12 of an additional peptide transport system. The range of substrates for that system was explored by seeing whether other peptides interfered with the growth response to trileucine.

The growth of both strains, TD-V and TD-V-TOR-2, on trileucine in the presence of trimethionine proceeded after a lag, whereas trilysine did not impede growth on trileucine in either strain (Fig. 1).

The Opp⁻ nature of all the TOR strains used was confirmed by the observation that in contrast to their parental strain (TD-V) they were unable to utilize Pro-Phe-Lys as a source of proline for growth and were resistant to the toxic tripeptides: trivaline and Lys_2-*p*-F-Phe.

Toxicity of trithreonine to E. coli K-12 TD-V and its Opp⁻ mutants. Since strain TD-V is auxotrophic for threonine, an attempt was made to use trithreonine to meet the threonine requirement. Surprisingly, the cells did not grow normally in the presence of the peptide even when supplemented with threonine. Trithreonine (80 μg/ml) suppressed the growth of strain TD-V in complete liquid medium (containing 50 μg of threonine per ml) for more than 10 h. Several Opp⁻ mutants of this strain (TD-V-TOR-2, -3, and -5) were similarly inhibited by trithreonine. As shown in Fig. 2, the lag in growth for strain TD-V-TOR-2 lengthened with increasing tripeptide concentration.

The inhibition of growth of strain TD-V-TOR-2 by trithreonine (0.105 mM) was antagonized by trileucine at a molar ratio of 1:1.1 (Thr_3-Leu_3), whereas trilysine at an even higher molar ratio (Thr_3-Lys_3) 1:2 did not affect the inhibition (Fig. 3). Trimethionine at a molar ratio of 1:2 did not affect trithreonine toxicity (data not shown). The sensitivity to trithreonine is not a general property of *E. coli* strains since trithreonine (100 μg/ml) did not affect the growth of *E. coli* W, *E. coli* TL3, and *E. coli* Bb.

Utilization of trithreonine by E. coli W TL3 and its Opp⁻ mutant. Since *E. coli* W did not display the same sensitivity to trithreonine as *E. coli* K-12, it was possible to directly test for trithreonine utilization in a W strain auxotrophic for threonine. *E. coli* W TL3 could utilize trithreonine (50 μg/ml) and trilysine (50 μg/ml) as sources of threonine and lysine, respectively (Fig. 4A). However, strain TL3-TOR as expected from its Opp⁻ nature, was unable to

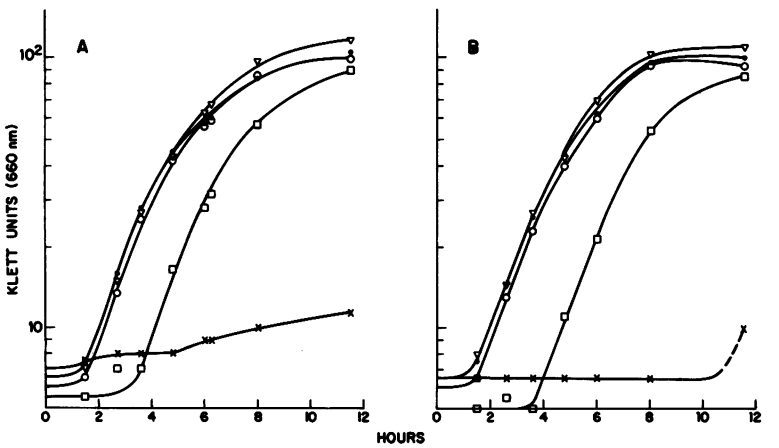

FIG. 1. *Inhibition of trileucine utilization in E. coli K-12 TD-V (A) and TD-V-TOR-2 (B). Growth conditions used are described in Materials and Methods. Symbols: (●) control growth in VB full Sm; (X) VB minus leucine Sm; (○) VB minus leucine Sm plus trileucine (50 μg/ml); (□) VB minus leucine Sm plus trileucine (50 μg/ml) plus trimethionine (230 μg/ml); (▽) VB minus leucine Sm plus trileucine (50 μg/ml) plus trilysine (300 μg/ml).*

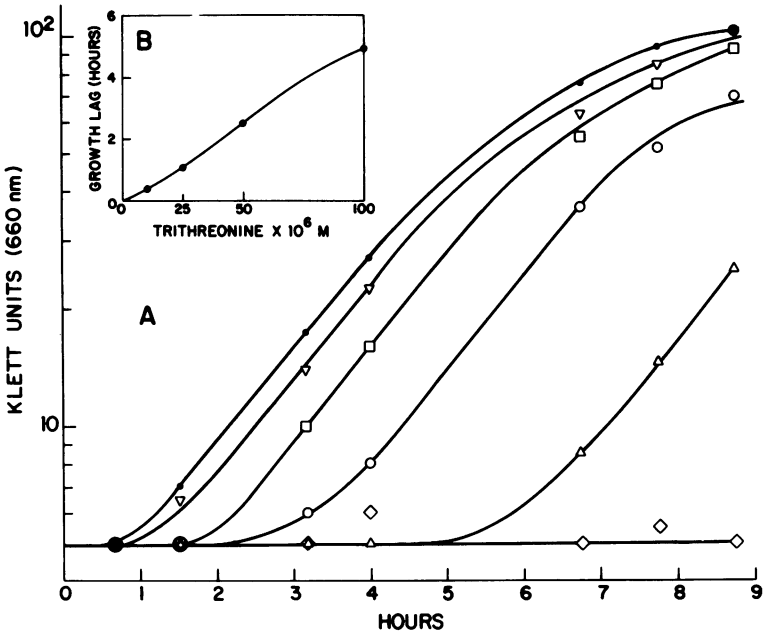

FIG. 2. *Inhibition of growth of strain TD-V-TOR-2 by various concentrations of trithreonine. Growth conditions used are described in Materials and Methods. Symbols:* (●) *control growth in VB full Sm. Trithreonine at various concentrations was added aseptically before inoculation to the other tubes as follows:* (▽) *10 μM;* (□) *25 μM;* (○) *50 μM;* (△) *100 μM;* (◇) *250 μM. Growth lag in part B was determined by the additional time required to reach turbidity of 10 Klett units (filter 660 nm) when compared to control.*

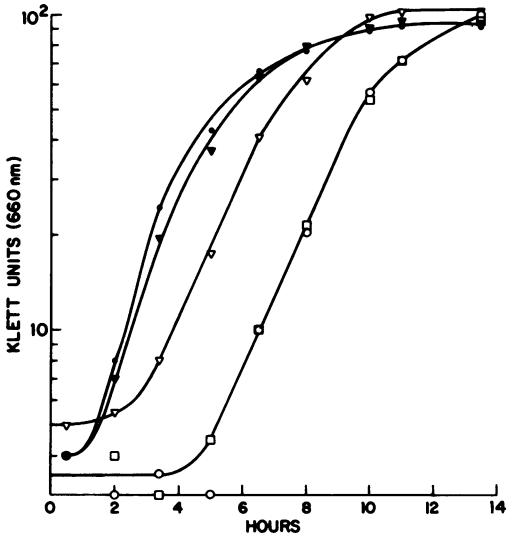

FIG. 3. *Competition of trileucine against trithreonine toxicity in strain TD-V-TOR-2. Growth conditions are described in Materials and Methods. Symbols:* (●) *control growth in VB full Sm;* (▼) *VB full Sm plus trileucine (0.14 mM);* (○) *VB full Sm plus trithreonine 0.105 mM;* (▽) *VB full Sm plus trithreonine 0.105 mM plus trileucine 0.11 mM;* (□) *VB full Sm plus trithreonine (0.105 mM) plus trilysine (0.2 mM).*

grow when trilysine was supplied as the sole source of lysine (Fig. 4B). The inactivity of the oligopeptide transport system in strain TL3-TOR was further confirmed by its resistance to the toxic tripeptides triornithine (Fig. 4B) and Lys_2-*p*-F-Phe (100 mM) (data not shown). Nevertheless, trithreonine (50 μg/ml) still supported exponential growth of the Opp^- mutant (Fig. 4B). Moreover, the growth of the Opp^- mutant on this tripeptide was similar to that observed with its parental strain - TL3. In both cases, trithreonine was only slightly less efficient than threonine.

The nutritional effectiveness of trithreonine in *E. coli* TL3-TOR could be antagonized by trimethionine at a molar ratio of 3:1 (Met_3-Thr_3) (Fig. 4B). However, trileucine at similar ratio did not compete with trithreonine utilization. Trivaline, which is transported exclusively by the oligopeptide transport system (Opp^- strains lose completely their ability to take in trivaline) also did not affect trithreonine utilization. Neither trimethionine (Fig. 4A) nor trileucine affected trithreonine-dependent growth in the Opp^+ strain of TL3.

Are trithreonine, trimethionine, and trileucine transported by the general oligopeptide transport system? The ability of tri-

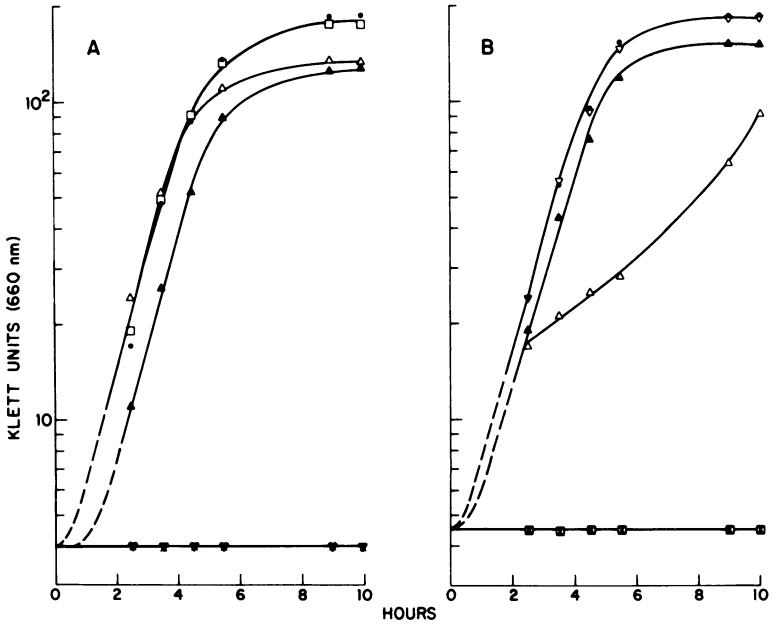

FIG. 4. *Peptide uptake by E. coli W TL3 (A) and TL3-TOR (B). Growth conditions are described in Materials and Methods. Symbols: (●) control growth in VB full; (X) VB minus threonine and VB minus lysine; (▽) VB full plus triornithine (100 μg/ml); (□) VB minus lysine plus trilysine (50 μg/ml); (▲) VB minus threonine plus trithreonine (50 μg/ml); (△) VB minus threonine plus trithreonine (50 μg/ml) plus trimethionine (170 μg/ml).*

threonine, trileucine, and trimethionine to utilize the Opp system was tested by measuring their ability to antagonize the growth inhibition of triornithine. This assay has the advantage that competition at the level of peptidases need not be considered since it is triornithine itself which is the inhibitory substance (2, 6).

The ability of the tripeptides to relieve the toxic effect of 25 μg of triornithine per ml (36 nmol/ml) towards strain M-26-26 was tested (Fig. 5). Competition experiments were carried out at molar ratios of 12 to 14:1 (competitor-Orn_3). All tripeptides tested antagonized triornithine toxicity. Peptides known to utilize the oligopeptide transport system exclusively, Pro-Phe-Lys (1) and trivaline, completely blocked triornithine toxicity and even slightly enhanced the growth as compared to the control. The other tripeptides (trileucine, trithreonine, and trimethionine) were less efficient, but in all cases showed strong competition. Trithreonine, trimethionine, and trileucine also competed against tetralysine utilization by the Opp^+ strain M-26-26 at a molar ratio of 10 to 11:1 (competitor-Lys_4). Trithreonine and trimethionine delayed the growth of the lysine auxotroph on tetralysine, increasing the lag period by 6 and 8 h, respectively. Trileucine was less efficient.

Isolation of spontaneous trithreonine-resistant mutants and studies of their peptide uptake systems. One of the ways to obtain resistance to a cytotoxic drug is to prevent its uptake. The toxicity of Thr_3 to strain TD-V provides us therefore with the means to select for mutants defective in their Thr_3 transport system. Spontaneous trithreonine-resistant (TTR) mutants were isolated from strains TD-V and TD-V-TOR-2 and designated as strains TD-V-TTR and TD-V-TOR-2-TTR, respectively. The isolation of the mutants was carried out from cultures that grew after a long lag in VB full Sm liquid medium containing 100 μg of trithreonine per ml. As demonstrated in Fig. 6 and Fig. 7, both TTR mutants are completely resistant to trithreonine. However, whereas the growth response of strain TD-V-TOR-2-TTR to trileucine was greatly reduced by the mutation (Fig. 7), strain TD-V-TTR showed no apparent change in its ability to utilize trileucine for growth (Fig. 6), when compared to its parental strain TD-V. The growth of strain TD-V-TOR-2-TTR in the presence of 56 μg of trileucine per ml as leucine source was poor and linear. Moreover, the rate of growth decreased and the lag was longer when the concentration of trileucine was lowered to 45 μg/ml (Fig. 7). Control experiments with both TTR strains

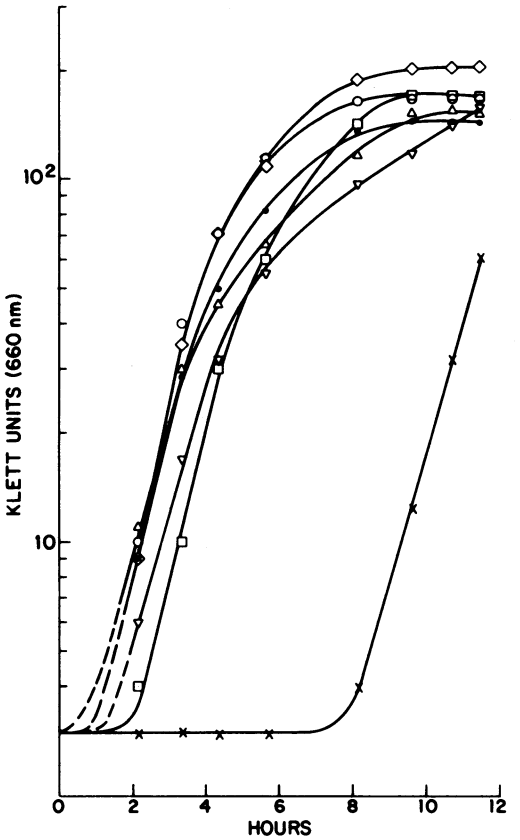

FIG. 5. *Competition of peptides against triornithine toxicity to strain M-26-26. Growth conditions used are described in Materials and Methods. Control cells were grown in VB full medium (●). All the other tubes contained VB full plus triornithine (25 μg/ml). Competitors were added as follows: (X) growth without competition; (▽) plus trileucine (150 μg/ml) (Leu_3-Orn_3 = 11.7:1); (□) plus trithreonine (150 μg/ml) (Thr_3-Orn_3 = 12.8:1); (△) plus trimethionine (200 μg/ml) (Met_3-Orn_3 = 13.3:1); (○) plus trivaline (150 μg/ml) (Val_3-Orn_3 = 13.3:1); (◇) plus Pro-Phe-Lys (200 μg/ml) (Pro-Phe-Lys:Orn_3 = 14.4:1).*

(TD-V-TTR and TD-V-TOR-2-TTR) revealed a normal growth response towards dipeptides. Pro-Phe was utilized normally as a proline source and divaline could effectively inhibit the growth of these mutants. In addition, strain TD-V-TTR was found to retain an active Opp system as it was able to utilize Pro-Phe-Lys and was sensitive to trivaline and Lys_2-*p*-F-Phe.

Both TTR strains were also able to utilize trithreonine as threonine source in VB minus threonine Sm medium. However, whereas strain TD-V-TTR gave a normal exponential growth response in the presence of 50 μg of trithreonine per ml (Fig. 6), strain TD-V-TOR-2-TTR responded poorly, giving a linear growth pattern (Fig. 7), somewhat similar to the one observed with trileucine.

DISCUSSION

In *E. coli*, an organism free of extracellular peptidases, growth response to peptides containing needed amino acids can serve as evidence for peptide uptake. Using the above experimental approach, the TOR mutants were shown to be altered in their oligopeptide transport system (1, 4). We confirmed these previous findings and secured additional evidence for the deficiency of oligopeptide transport in the TOR mutants studied in this report. Specifically, *E. coli* K-12 TD-V-TOR-2, in contrast to its parental strain TD-V, was unable to utilize Pro-Phe-Lys as proline source and was resistant to the cytotoxic tripeptides, triornithine, Lys_2-*p*-F-Phe, and trivaline, and strain *E. coli* W TL3-TOR could not utilize tri- and tetralysine as lysine sources and was resistant to triornithine and Lys_2-*p*-F-Phe. However, in spite of

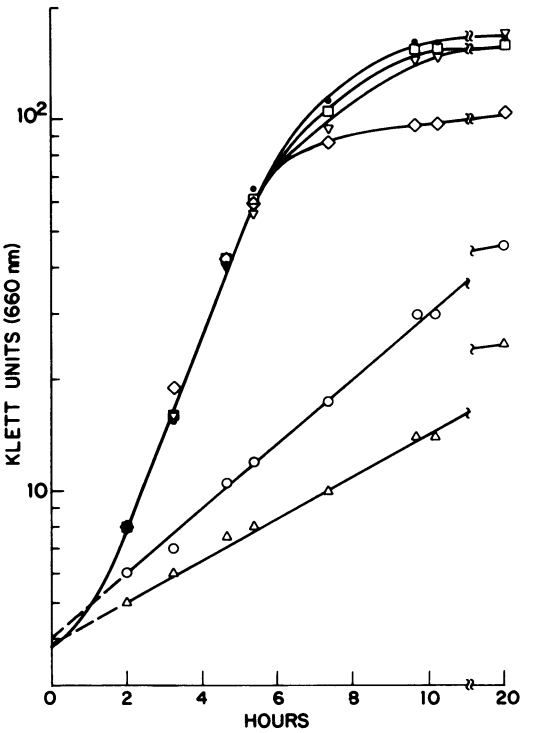

FIG. 6. *Toxicity and utilization of peptides by strain TD-V-TTR. Growth conditions used are described in Materials and Methods. Symbols: (●) control growth in VB full Sm; (▽) VB minus leucine Sm plus trileucine (50 μg/ml); (□) VB minus threonine Sm plus trithreonine (50 μg/ml); (◇) VB minus proline Sm plus Pro-Phe-Lys (50 μg/ml); (○) VB full Sm plus trivaline (50 μg/ml); (△) VB full Sm plus divaline (55 μg/ml).*

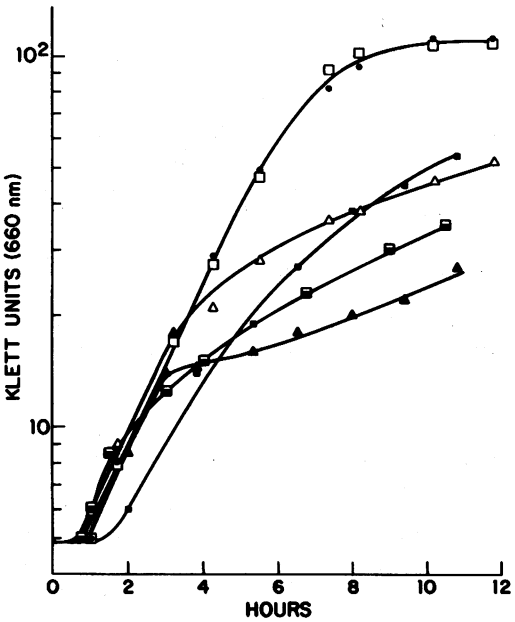

FIG. 7. *Trileucine and trithreonine utilization by strain TD-V-TOR-2-TTR. Growth conditions are described in Materials and Methods. Symbols:* (●) *control growth in VB full Sm;* (□) *VB full Sm plus trithreonine (100 μg/ml);* (■) *VB minus threonine Sm plus trithreonine (50 μg/ml);* (▣) *VB minus threonine Sm plus trithreonine (35.5 μg/ml);* (▲) *VB minus leucine Sm plus trileucine (45 μg/ml);* (△) *VB minus leucine Sm plus trileucine (56 μg/ml).*

their oligopeptide transport deficiency, both Opp⁻ strains could still take up a number of tripeptides. For example, trileucine could support exponential growth of several Opp⁻ strains of *E. coli* K-12 TD-V. The effectiveness of this tripeptide in supporting the growth of the leucine auxotrophs was similar in both Opp⁺ and Opp⁻ strains, indicating retention of trileucine transport in the absence of an active Opp system. This finding that trileucine is taken up through a peptide transport system other than the Opp, accords with the failure of trilysine, which is known to utilize the Opp system exclusively (16), to compete with trileucine-supported growth in strain TD-V. Trimethionine, however, competed with trileucine utilization, indicating that it too could be recognized by the second transport system.

Another tripeptide that was found to be transported into Opp⁻ mutants was trithreonine. This was demonstrated with *E. coli* W as well as with *E. coli* K-12 strains. Specifically, *E. coli* W TL3-TOR yielded exponential growth, similar to that obtained with the Opp⁺ strain of TL3 when trithreonine was supplied as the sole source of the required threonine. Moreover, this growth response of the Opp⁻ strain to trithreonine was not affected by trivaline, a substrate that only uses the general oligopeptide transport system. It seems, therefore, that trithreonine is taken into *E. coli* W TL3 via a new transport system distinct from the general oligopeptide transport system Opp. In *E. coli* W trimethionine is also recognized by this second system, as in contrast to trivaline it inhibited the growth of strain TL3-TOR on trithreonine.

The conclusion that trithreonine utilizes an additional transport system was further supported by the observation that trithreonine, a growth inhibitor of *E. coli* K-12 TD-V, retained its toxicity towards the Opp⁻ mutants TD-V-TOR-2, -3, and -5. No significant difference in the degree of sensitivity was observed when comparing the Opp⁻ strains with their parental strain TD-V. A possible difficulty with this interpretation is that the site of action of trithreonine could have been on the cell surface. However, findings with *E. coli* W TL3-TOR, discussed above, have already indicated uptake of trithreonine by an Opp⁻ mutant. Moreover, the toxicity of trithreonine could be antagonized by other peptides (trileucine and trimethionine) that were shown to be taken up by Opp⁻ mutants of strain TD-V (see Results). Furthermore, resistance to trithreonine in strain TD-V-TOR-2-TTR resulted from an alteration of the trithreonine-trileucine transport system, as it was accompanied by a strong reduction in the capability of strain TD-V-TOR-2 to utilize trileucine as leucine source. It can be concluded, therefore, that Opp⁻ mutants of strain TD-V are able to accumulate trithreonine inside their cells.

The observed uptake of trileucine and trithreonine might have been mediated by diffusion rather than via a transport system. However, the possibility of diffusion is unlikely since the uptake needed for normal growth did not require high concentrations of substrate in the medium and competition phenomena among the peptide substrates of the second system could be demonstrated.

Transport of trithreonine and trileucine into *E. coli* TOR mutants is explained most simply by uptake through an additional transport system which is distinct from the Opp system. An alternative possibility is that Opp⁻ mutants retain residual transport activity because of a change in the Opp system itself. However, the latter postulation seems unlikely because no competition against either trithreonine or trileucine uptake in the Opp⁺ strains *E. coli* K-12-TD-V and *E. coli* W TL3 was observed when using trilysine or trivaline as competitors. Trilysine was shown to possess high affinity towards the Opp system (16) and both it and

trivaline were shown to utilize the Opp system exclusively. These peptides should affect, at least to some degree, any transport that is exclusively dependent on the Opp. Furthermore, the Opp$^-$ mutations in *E. coli* TD-V-TOR-2, -3, and -5 were identified as deletions and very probably completely lack the Opp system, again ruling out the possibility of retention of a residual uptake through a mutated transport system in the TOR strains. It can be concluded, therefore, that the uptake of trithreonine and trileucine can be carried out by a transport system distinct from the Opp.

Lastly, it was shown that the peptides trithreonine, trimethionine, and to a lesser extent trileucine, are also transported by the general oligopeptide transport system, as they all relieved the toxicity of triornithine towards strain M-26-26 and competed against tetralysine utilization as lysine source by this lysine-required strain. Inhibition of the utilization of a peptide for growth might be due to competition either at the level of intracellular peptidases or transport. However, relief of triornithine toxicity by peptides cannot be explained by competition at the level of peptidase, since triornithine itself rather than its degradation products is the toxic agent. The competition achieved in both of the instances (discussed above) is explained most simply therefore, as inhibition of uptake. The ability of trithreonine to utilize the Opp system is in agreement with the difficulties we encountered in attempting to obtain a spontaneous TTR strain that would be altered in its second oligopeptide transport system. The only type of TTR strain obtained when selecting from the Opp$^+$ strain TD-V, gained resistance to trithreonine through a mechanism other than prevention of uptake, as no change was observed in the mutants' ability to utilize trileucine as leucine source. These difficulties can be explained by the low probability of isolating strains requiring mutations in two independent systems. However, the use of an Opp$^-$ mutant (TD-V-TOR-2) as parental strain eliminated one of the two uptake systems and allowed selection for a TTR mutant that was altered in its trithreonine uptake.

It is of considerable interest that all of the peptides that were shown to be transported into *E. coli* by the second transport system were also substrates of the Opp. It would appear that the oligopeptide permease serves as a generalized system for peptide uptake and in addition a more specific system (or systems), such as the one mentioned above, also exists. This situation somewhat resembles that found for several amino acids that may be taken into the cell by transport systems, both with broad and narrow specificities. However, as the range of possible substrates for this specialized system has not yet been determined, one cannot anticipate the significance and the role this transport system plays in the overall peptide uptake in *E. coli*.

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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., S. Sarid, and E. Katchalski. 1973. Inhibition of protein biosynthesis in *Escherichia coli* B by tri-L-ornithine. Eur. J. Biochem. **34**:317-324.
3. De Felice, M., J. Guardiola, A. Lamberti, and M. Iaccarino. 1973. *Escherichia coli* K-12 Mutants altered in the transport systems for oligo- and dipeptides. J. Bacteriol. **116**:751-756.
4. Fickel, T. E., and C. Gilvarg. 1973. Transport of impermeant substances in *E. coli* by way of oligopeptide permease. Nature (London) New Biol. **241**:161-163.
5. Gilvarg, C. 1972. Peptide transport in bacteria, p. 11. *In* Peptide transport in bacteria and mammalian gut, Ciba Foundation Symposium, Elsevier, Excerpta Medica, North Holland. Associated Scientific Publishers, Amsterdam.
6. Gilvarg, C., and Y. Levin. 1972. Response of *Escherichia coli* to ornithyl peptides. J. Biol. Chem. **247**:543-549.
7. Gilvarg, C., and E. Katchalski. 1965. Peptide utilization in *Escherichia coli*. J. Biol. Chem. **240**:3093-3098.
8. 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.
9. 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.
10. Payne, J. W. 1971. The utilization of prolyl peptides by *Escherichia coli*. Biochem. J. **123**:255-260.
11. Payne, J. W. 1972. Effects of N-methylpeptide bonds on peptide utilization by *Escherichia coli*. J. Gen. Microbiol. **71**:259-265.
12. Payne, J. W. 1972. Mechanism of bacterial peptide transport, p. 17. *In* Peptide transport in bacteria and mammalian gut. Ciba Foundation Symposium, Elsevier, Excerpta Medica, North Holland. Associated Scientific Publishers, Amsterdam.
13. Payne, J. W. 1973. Peptide utilization in *Escherichia coli*: studies with peptides containing β-alanyl residues. Biochim. Biophys. Acta **298**:469-478.
14. Payne, J. W. 1974. Peptide transport in *Escherichia coli*: permease specificity towards terminal α-amino group substituents. J. Gen. Microbiol. **80**:269-276.
15. Payne, J. W., and C. Gilvarg. 1968. The role of the terminal carboxyl group in peptide transport in *Escherichia coli*. J. Biol. Chem. **243**:335-340.
16. Payne, J. W., and C. Gilvarg. 1971. Peptide transport. Adv. Enzymol. **35**:187-244.
17. Simmonds, S. 1972. Peptide activity and peptide metabolism in *Escherichia coli*, p. 43. *In* Peptide transport in bacteria and mammalian gut, Ciba Foundation Symposium, Elsevier, Excerpta Medica, North-Holland. Associated Scientific Publishers, Amsterdam.
18. Sussman, A. J., and C. Gilvarg. 1970. Peptide transport and metabolism in bacteria. Annu. Rev. Biochem. **40**:397-408.
19. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97-106.