Oligopeptide Transport in *Escherichia coli*

SPECIFICITY WITH RESPECT TO SIDE CHAIN AND DISTINCTION FROM DIPEPTIDE TRANSPORT*

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SUMMARY

A lysine auxotroph of *Escherichia coli* W can use lysine oligopeptides as a source of the required amino acid. A mutant, defective in oligopeptide transport, was isolated from this strain with the use of resistance to the bactericidal action of triornithine as a selection procedure. The triornithine-resistant mutant could no longer grow upon lysine oligopeptides. In an analogous manner a triornithine-resistant mutant of a glycine auxotroph lost the ability to grow upon glycine oligopeptides. It is concluded that lysine, ornithine, and glycine oligopeptides use a common transport system, which is lost in the triornithine-resistant strain. This conclusion is substantiated by studies that show competition among these oligopeptides for entry into the cell. Tyrosine oligopeptides can also compete, and they are presumed to use the same transport system. This lack of specificity with respect to side chain is taken as an indication that a single system may be used for oligopeptide transport and therefore is in marked contrast to the transport of the free amino acids, for which several distinct systems are required. It is shown that dipeptides use predominantly a separate system, although they possess limited ability to enter by the oligopeptide mechanism. In contrast, oligopeptides cannot be transported by the specific dipeptide system or systems.

Previous reports from this laboratory have been concerned with attempts to characterize the system responsible for peptide transport in *Escherichia coli*. In these studies, lysine peptides were chosen as model compounds, and it was shown that, for an oligopeptide to be transported, the presence of a free α-amino group is essential (1, 2), but the terminal carboxyl group is not vital (3). The isolation of a mutant that had lost the ability to transport certain oligopeptides but retained its normal ability to transport a number of dipeptides indicated that separate transport systems were used by the two classes (3). Evidence was presented to suggest that the dipeptide transport system or systems are distinct from the oligopeptide transport system or systems in requiring not only a free α-amino group but also a free terminal carboxyl group (3). The fact that higher homologues of several oligopeptide series are unable to enter *E. coli* was correlated with a critical size above which an oligopeptide is unable to traverse the cell envelope (2, 3).

The work reported here details an investigation into the specificity requirements with respect to amino acid side chains of the system responsible for oligopeptide transport. The structurally dissimilar lysine, glycine, and tyrosine oligopeptides were selected for study. The three amino acids are transported by distinct systems, but in contrast the results here indicate that the oligopeptides are able to utilize a common transport system. This is shown in two ways. First, it is shown that a strain selected for its inability to transport a specific oligopeptide simultaneously loses its ability to transport a variety of other oligopeptides. In the specific case here, it is shown that glycine and lysine auxotrophs can use glycine and lysine oligopeptides as sources of the required amino acids, but when substrains are selected that are resistant to the toxic oligopeptide triornithine, the mutation involved is pleiotropic; the cells can no longer grow upon those oligopeptides that previously were nutritionally active. Second, with the use of both wild type and auxotrophic strains it is shown that these oligopeptides compete with one another for entry into the cell.

Although the various oligopeptides studied can use a single transport system, they possess significant differences in their affinities for this transport system. It is shown that in addition to the general oligopeptide transport system there exists some other facility by which oligopeptides can enter the cell, but that entry by this latter mechanism is not susceptible to competition by other oligopeptides. Certain oligopeptides that are unable to enter *E. coli* (1–3) also fail to act as competitive inhibitors for

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oligopeptides that are known to enter. To exhibit competitive activity a peptide must possess a free \(\alpha\)-amino group. Further information concerning the nature of the separate transport systems for di- and oligopeptides is presented.

**EXPERIMENTAL PROCEDURE**

**Materials**

Oligopeptides—The lysine oligopeptides were prepared by the acid hydrolysis of poly-L-lysine (Pilot Chemicals, Watertown, Massachusetts) as described previously (3). The synthesis of \(\alpha\)-acetyl trilysine was reported earlier (1). Oligopeptides containing ornithine residues were a gift from Dr. Y. Levin of the Massachusetts Institute. Trityrosine was purchased from Miles Laboratories; glycine peptides were purchased from Cyclo Chemical Corporation, Los Angeles, California; and all other peptides were obtained from Mann.

**Methods**

Peptide Assays—The concentrations of lysine oligopeptides were determined upon total hydrolysates by means of the ninhydrin assay for lysine described by Shimura and Vogel (4). The results were checked with a biological assay with the use of a lysine auxotroph of E. coli. These procedures have been detailed previously (3).

Assay of Peptidase Activity—Cells were harvested at the cessation of exponential growth and broken with a Branson Sonifier. The cleavage of triglycine and trilylsine was measured in incubation mixtures that contained 100 \(\mu\) M potassium phosphate buffer (pH 7.1), crude extract corresponding to 0.3 mg of protein, and peptide or peptides in a final volume of 1.0 ml. In controls, triglycine (2.0 \(\mu\) moles per ml) or trilylsine (0.04 to 0.4 \(\mu\) mole per ml) was added separately, and in competition studies both were present. Incubation was at 37°. The reaction was stopped by the addition of HCl, and after centrifugation, portions of the supernatant solutions were assayed for ninhydrin-positive cleavage products by the procedure of Moore and Stein (5).

For electrophoretic investigation of peptidase action, samples from the above supernatant solutions were applied to carboxymethyl cellulose paper, Whatman No. CM-U. Electrophoresis was carried out in pyridine acetate buffer (pH 3.5), prepared from the above supernatant solutions were applied to carboxymethyl cellulose paper, Whatman No. CM-U. Electrophoresis was carried out in pyridine acetate buffer (pH 3.5), prepared from glacial acetic acid-pyridine-water (10:1:89 v/v). Samples were run at 20° at 80 volts per cm for 30 min. After electrophoresis the papers were dried, then dipped in a solution of ninhydrin (0.5% w/v) in 95% aqueous acetone, and developed by heating.

Bacterial Strains—The following strains of E. coli W (ATCC 9637) were used in addition to the wild type. Strain M-20-26 is a lysine auxotroph that lacks the deacarboxylase that converts meso-diaminopimelic acid to L-lysine (6, 7). A mutant was isolated from strain M-26-26 by the use of resistance to the bactericidal action of triornithine as a selection procedure (3).\(^2\) The triornithine-resistant mutant was designated M-26-26.TOR. The resistance arises from the loss of a functional transport system to transport triornithine into the cell. That strain M-26-26, TOR has this deficiency in peptide transport is inferred from the fact that the TOR mutant no longer has the ability, possessed by the parental strain M-26-26, to grow upon lysine oligopeptides (3).

Strain M-22-93, a glycine-serine auxotroph, was obtained from Dr. W. Maas. The strain was found to be somewhat heterogeneous in its growth response to glycine, and the substrain showing the fastest growth rate was isolated and used here; it was designated M-123. From strain M-123 a mutant was isolated by means of resistance to the bactericidal action of triornithine as a selection procedure. The triornithine-resistant mutant was designated M-123.TOR. The bacterial cultures were grown in the Medium A of Davis and Mingioli (8). Growth media and peptides were sterilized by autoclaving at 15 pounds of pressure for 15 min. Sterile glucose solution (25%) was added aseptically after cooling. Growth was studied by measuring absorbance in a Klett-Summerson colorimeter with a 660 nm filter. For competition experiments with auxotrophs, inocula were taken from cultures grown on limiting amounts of the appropriate amino acid that give a final absorbance of 50 Klett units. When exponential growth ceased at 50 Klett units, the cultures were incubated for an additional 60 to 90 min to exhaust endogenous supplies completely of the essential amino acid. Portions (0.5 ml) were then used for inoculating the test solutions (9.5 ml). All studies with the glycinolysine auxotrophs were carried out with the use of nephelo flasks with aeration. With strain M-26-26, growth was carried out with Klett tubes. When wild type E. coli was used, inocula (0.1 ml) were removed from a culture growing exponentially on minimal media and added to the test solutions (4.9 ml) in Klett tubes. Growth in Klett tubes was carried out without agitation. All cultures were incubated at 36°.

**RESULTS**

Growth Characteristics of Glycine Auxotroph—In previous investigations lysine peptides were used as model compounds. For the present study, however, glycine peptides were selected. Since these peptides have the simplest of all amino acid side chains, their use provides a great contrast to the studies on the lysine peptides in which the side chains are both bulky and charged. A convenient way to study peptide transport is to measure the ability of an appropriate peptide to meet the growth requirement of an amino acid auxotroph. In the present case, the growth response of a glycine auxotroph grown in minimal media supplemented with glycine peptides was studied. It is useful to carry out a preliminary assessment of the response to glycine itself. In Fig. 1 is shown a calibration curve for the growth response of a glycine auxotroph, M-123, as a function of the concentration of glycine in the media. There is a linear response at first. However, the curve levels off at the higher glycine concentrations because in the very dense cultures the buffering capacity of the media has been exhausted by the acid produced during growth.

Having established the pattern of response to glycine, one can determine the response to glycine peptides. In Fig. 2 are shown the growth patterns of strain M-123 for the glycine oligopeptides up to the hexamer in size. Growth was carried out in the presence of equimolar concentrations of the oligopeptides. It can be seen that growth is obtained in all cases. Relatively high concentrations of oligopeptides (in terms of glycine equivalents) are required in the case of the larger oligopeptides to sustain an adequate rate of growth. It was found that with the use of low concentrations (approximately 0.8 \(\mu\) moles of glycine per ml) the over-all growth rate was slower with the larger oligopeptides (not shown), this being particularly noticeable toward the end of growth. The slow growth rate seen with diglycine was
unexpected. However, diglycine is not itself inhibitory, for it does not interfere with the growth of strain M-123 on media supplemented with glycine. It was found that growth upon limiting amounts (i.e., less than 0.8 \( \mu \text{eq} \) of glycine per ml) of tri- or tetraglycine continued exponentially until the complete utilization of the oligopeptides. The complete cleavage of these oligopeptides must at some stage proceed via the dipeptide. It is likely, therefore, that the slow growth upon diglycine is caused by the limiting rate of its entry rather than by the inadequate rate of its cleavage.

**Mutants Deficient in Oligopeptide Transport**—The possibility of using mutants deficient in oligopeptide transport as a probe into the side chain specificities of the oligopeptide transport system stemmed from an earlier observation that triornithine exerts a bactericidal effect toward *E. coli* (3). It was apparent that among the several possible classes of triornithine-resistant organisms, there should exist one in which the ability to transport the tripeptide into the cell has been lost. The cell could become triornithine resistant in several other ways, for example, by the acquisition of such enhanced levels of peptidase activity as to prevent the accumulation of toxic quantities of triornithine within the cell, or by a mutation providing increased resistance at the specific site sensitive to the tripeptide. In practice, however, mutants that lack the ability to transport triornithine have proved the easiest to isolate. In previous studies, it was shown that M-26-26, a lysine auxotroph of *E. coli*, could use lysine oligopeptides as a source of the required amino acid (2). However, a triornithine-resistant mutant, M-26-26.TOR, isolated from this strain could no longer grow upon lysine oligopeptides (3). It was therefore suggested that, in this mutant strain, triornithine resistance arose from a derangement of the transport system for taking triornithine into the cell and that this system was used by both lysine and ornithine oligopeptides. It would be expected that the structurally similar lysine and ornithine oligopeptides might use the same transport system, but the question arose as to whether in fact this system might not be able to handle a great variety of oligopeptides, in contrast to the diversity of systems required for the amino acids. The study of triornithine-resistant strains of other amino acid auxotrophs appeared to offer a way of answering this question.

The growth of strain M-123 is inhibited by triornithine in a manner identical with that described previously for strain M-26-26 (3). A spontaneous mutant resistant to triornithine was isolated and tested for its ability to use glycine oligopeptides. In Fig. 3 it is shown that this mutant, M-123.TOR, is unable to utilize glycine oligopeptides. Under identical growth conditions, the parental strain M-123 grows equally well upon glycine, triglycine, or tetraglycine. The result is analogous to that described above for M-26-26.TOR (3). It appears, therefore, that a mutation resulting in an inability to transport triornithine also prevents the transport of glycine and lysine oligopeptides. These oligopeptides must utilize some common feature of a transport system that is either absent or nonfunctional in the triornithine-resistant strain. Because it is not possible with these monoauxotrophic strains to measure the transport of both glycine and lysine oligopeptides with a single TOR mutant, the above conclusion necessarily rests upon the assumption that the mutations in the two independently isolated TOR strains are functionally identical. Further support for the conclusion is provided by the results of competition experiments described later.

In the same experiment (Fig. 3) it was observed that the growth response to diglycine is identical in both strain M-123 and M-123.TOR. This result is analogous to that found with strain M-26-26 and M-26-26.TOR, both of which respond identically with dilysine (3). Furthermore, heterologous dipeptides containing glycine, such as glycyl-l-serine, produced identical growth responses in the two glycine strains. This again resembles earlier results obtained with the lysine auxotrophs grown upon heterologous dipeptides containing lysine (3).

**Competition Leading to Growth Inhibition**—If structurally diverse oligopeptides do enter *E. coli* by a single transport
system, it would be expected that different oligopeptides would compete with one another for entry into the cell. This possibility can be conveniently tested in the following way. If an amino acid auxotroph were to be grown in the presence of an oligopeptide that contained the essential amino acid as a residue, then competition for entry by another oligopeptide that lacks the required amino acid should deprive the auxotroph of its essential nutrient and thus cause inhibition of growth. The use of several auxotrophs should allow one to investigate in detail the reciprocal nature of such oligopeptide competition. However, it is first necessary to establish the relative amounts of competitor required for inhibition to be detectable. In Fig. 4 is shown the effect of various concentrations of trilysine upon the growth response of strain M-123 to tetraglycine. At a molar ratio of tetraglycine-trilysine of 50:1, an initial lag in growth is observed, and it is prolonged with increasing amounts of trilysine. At a molar ratio of 100:1, inhibition is barely detectable. At these concentrations of trilysine the inhibitory effect is not permanent, for following an initial lag period, growth proceeds at a rate equal to that observed for the control without added trilysine. Trilysine is not itself inhibitory, for it does not interfere with the growth of strain M-123 on media supplemented with glycine. These results are consistent with a process involving the initial, preferential uptake of the small amounts of trilysine and its removal by intracellular peptidase activity, which then allows the uninhibited entry of tetraglycine. When strain M-123 was grown in the presence of triglycine, slightly higher amounts of trilysine were required for inhibition; however, a lag period could readily be detected at a molar ratio of triglycine-trilysine of 25:1.

In Fig. 5 is shown the result of the reciprocal experiment in which the inhibitory effect of triglycine upon the growth of strain M-26-26 upon trilysine is examined. As anticipated from the results shown in Fig. 4, high concentrations of glycine oligopeptides are required to limit the growth upon lysine oligopeptides. At a molar ratio of triglycine-trilysine of 50:1, no inhibition is observed; but, at a molar ratio of 100:1, a reduction in the growth rate is observed. In contrast to the initial short lag period that is observed with the glycine auxotroph (Fig. 4), with the lysine auxotroph a fairly normal rate of growth is seen initially, but this is followed by a permanent inhibitory effect. This result is consistent with a process by which trilysine, at a molar ratio relative to triglycine of 1:100, can initially enter the cell at a rate sufficient to support growth.

Fig. 3. Growth of strain M-123.TOR and M-123 on glycine peptides. Glycine and glycine peptides were all used at a concentration of 1.0 μmole of glycine or glycine residues per ml. Triornithine was used at a concentration of 0.04 μmole per ml. , strain M-123.TOR; --- - , strain M-123.TOR and M-129.

Fig. 4. The effect of trilysine on the growth response of strain M-123 to tetraglycine. Numbers refer to concentrations of oligopeptides in micromoles per ml. Tetraglycine was present in all cases at a concentration of 0.3 μmole per ml.

Fig. 5. The effect of triglycine on the growth response of strain M-26-26 to trilysine. Numbers refer to concentrations of oligopeptides in micromoles per ml. Trilysine was present in all cases at a concentration of 0.033 μmole per ml.
However, the slow entry of triglycine and its consequent slow removal would lead to its continued presence in an ever increasing excess over the trilysine in the media, which finally would give rise to a prolonged competitive, and thus inhibitory, effect. Triglycine itself is not inhibitory at the high concentrations used in Fig. 5, for it does not alter the growth of strain M-26-26 in media containing lysine.

It appeared feasible to use structurally diverse oligopeptides as competitors in the above test systems and thus use the procedure to assess the specificity of the oligopeptide transport system or systems. Trityrosine was selected as representative of a type of oligopeptide structurally dissimilar to either lysine or glycine oligopeptides. In Fig. 6 it can be seen that at approximately equimolar concentrations of trityrosine and tetraglycine a marked inhibition of growth of strain M-123 on the tetraglycine is observed. Trityrosine caused no inhibition in the growth of strain M-123 on glycine. On the assumption that the three types of oligopeptides enter predominantly by a single oligopeptide transport mechanism, it would appear from the previous observations on the relative amounts of glycine oligopeptides required to inhibit the growth of strain M-26-26 on trilysine (Figs. 4, 5), together with the above relationship between trityrosine and tetraglycine, that molar ratios of trityrosine-trilysine of 50 to 100:1 would be required for competition between the two to be observed. In Fig. 7 it is seen that inhibition of growth of strain M-26-26 on trilysine cannot be observed until, as expected, the molar ratio of trityrosine-trilysine is of the order of 50:1. It would seem from these results that a detailed extension of such studies should allow one to measure the relative affinities of different oligopeptides for the transport system.

**Competition Leading to Growth Enhancement**—It was important to establish that the growth inhibitory effects described above are not a consequence of the auxotrophic nature of the strains studied, in which inhibition of peptidase activity could curtail growth. This was studied in two ways. First, competition for entry was tested with the use of wild type E. coli, in which growth is not directly dependent on peptidase activity. Wild type cells exposed to triornithine are killed. However, any oligopeptide that can prevent triornithine from entering the cell should be able to overcome the usual bactericidal effect of triornithine. Competition in this system, therefore, should be manifested in an enhanced growth response. Lysine oligopeptides were selected as competitors, and as shown in Fig. 8, tri- and tetralysine are both extremely effective in overcoming the inhibitory effects of triornithine, growth commences almost immediately in contrast to the approximate 10-hour lag with triornithine alone. Dilysine showed significant, although inferior, competitive ability in this system. Lysine itself was without effect.

**Competition and Peptidase Activity**—Second, to provide additional evidence that the growth-inhibitory effects observed with the auxotroph do not arise from inhibition of peptidase activity, direct measurements were made of the effect of trilysine on the cleavage of triglycine by crude extracts of E. coli. In the presence of a molar ratio of trilysine to triglycine 10 times...
In previous studies it was shown that for an oligopeptide to be transported into *E. coli* the presence of a free α-amino group is essential (1, 2). If the oligopeptide transport system were to possess several distinct components, the possibility could arise that this requirement for an α-amino group could be manifested by a component different from that which constitutes the principal site of the competitive action described above. To investigate this possibility the ability of α-acetyl trilysine to compete with glycine oligopeptides was studied. In Fig. 9 it can be seen that trilysine, at a concentration of 0.006 μmole per ml, inhibits the growth of M-123 on tetraglycine; however, α-acetyl trilysine at 10 times this concentration is without effect. It therefore appears either that the site of competition itself requires an oligopeptide to possess a free α-amino group or that an oligopeptide that lacks such a group cannot reach the site of competition.

Peptide Entry by Secondary Mechanism—The growth response of the triornithine-resistant mutant, M-123.TOR, to several heterologous tripeptides containing glycine residues was also tested. It was observed in all cases that a peptide concentration sufficient to give exponential growth in strain M-123 gave essentially no growth with mutant M-123.TOR. However, it was found in several cases that by increasing the peptide concentration some growth of strain M-123.TOR could be obtained. The growth rate increased slightly in response to further increases in peptide concentration but remained essentially linear. Exponential growth was never achieved. These results raised the question as to whether the TOR mutation produced an impaired but not totally unfunctional transport system or whether peptides could enter the cell by some means in addition to the oligopeptide transport system described above. If the TOR mutant possesses an impaired system, it would be expected that competition might still be observed, but, if entry can also occur

Table 1

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<th>Observed optical density at 570 mp after incubation for various times</th>
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that at which a significant inhibition of growth of strain M-123 is observed, there was no inhibition of cleavage of triglycine. This was shown initially in a qualitative manner by the use of electrophoresis. In this way it could be seen visually that the production of glycine and diglycine was the same in the presence or absence of trilysine. It was then shown quantitatively, by means of the assay of Moore and Stein (5), that the formation of ninhydrin-positive material in an incubation mixture containing both peptides was the sum of that obtained when trilysine and triglycine were treated separately. These data are shown in Table 1.

Essentiality of Free α-Amino Group for Competitive Activity—

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![Figure 8](http://www.jbc.org/)

**FIG. 8.** The ability of lysine peptides to overcome the inhibitory effect of triornithine in wild type *E. coli*. Except as shown, triornithine was present in all cases at a concentration of 0.04 μmole per ml. Numbers refer to concentrations of amino acid or peptides in micromoles per ml.

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![Figure 9](http://www.jbc.org/)

**FIG. 9.** The relative effects of trilysine and α-acetyl trilysine on the growth response of strain M-123 to tetraglycine. Numbers refer to concentrations of oligopeptides in micromoles per ml. Tetraglycine was present in all cases at a concentration of 0.3 μmole per ml.
by some nonspecific process, then competition is unlikely. Furthermore, if entry can occur also by a secondary process, it would be expected that by competitive inhibition it should not be possible to reduce the rate of growth of strain M-123 to below that observed with strain M-123.TOR. These possibilities were tested with the use of glycyl-L-leucylglycine as a source of glycine and trilysine as a competitor. It is seen in Fig. 10 that when strain M-123 is grown on glycyl-L-leucylglycine the addition of increasing amounts of trilysine causes a proportional reduction in the growth rate to a level approaching that seen with mutant M-123.TOR. At higher concentrations of trilysine the limiting rate of growth shown by strain M-123.TOR is reached. Trilysine is not itself inhibitory, for the same concentrations do not impair the growth of strain M-123 on glycine-supplemented media. Furthermore, the growth rate of strain M-123.TOR is unaffected by the presence of trilysine, indicating that the process by which glycyl-L-leucylglycine enters this mutant is not susceptible to competition. It appears most likely, therefore, that in strain M-123.TOR the entry of oligopeptides is not by an impaired version of the specific oligopeptide transport system being considered here, but by some other mechanism.

Characteristics of Dipeptide Transport—Finally, the nature of the distinct dipeptide transport system or systems was studied. As shown in Fig. 2, the rate of growth of strain M-123 on diglycine is significantly less than that upon either glycine or the higher oligopeptides at equal molarities. When tested at a concentration of 0.5 μmole per ml, a variety of dipeptides, including glycyl-L-aspartic acid, glycyl-L-leucine, L-phenylalanylglycine and glycyl-DL-valine, resembled diglycine in their poor ability to support the growth of strain M-123. In contrast, glycyl-DL-serine was found to support exponential growth at this concentration. Nevertheless, strain M-123 contained enzymatic activity able to cleave all these dipeptides. However, as shown in Fig. 11, by increasing the concentration of diglycine, the growth rate of strain M-123 can be increased. The fact that dilysine inhibits growth under these conditions to give a slow, linear rate of growth implies that the entry of diglycine into the cell is susceptible to competition and is therefore most likely to occur by a specific transport system.

From the existence and nature of the TOR mutation, it would appear that oligopeptides enter E. coli by a system distinct from that used by dipeptides, and it would therefore be expected that oligopeptides should be unable to inhibit competitively the uptake of dipeptides. To test this assumption, the effect of trilysine upon the growth response of strain M-123 to triglycine was measured. Glycyl-DL-serine was supplied to both strains at a concentration of 1.0 μmole per ml. The addition of trilysine at concentrations up to
1.1 μmol per ml caused no diminution in growth rate in either strain. On the other hand, it has been suggested on the basis of other studies (3) that the converse situation can occur, i.e., dipeptides have some ability to enter the cell through the oligopeptide transport system. This should therefore be manifested in a competitive inhibition of oligopeptide uptake by dipeptides. To investigate this aspect, the effect of dilysine upon the growth response of strain M-123 to triglycine was studied. It can be seen in Fig. 12 that, as anticipated, the addition of an approximately equal molar amount of dilysine severely depresses the growth rate from that observed with triglycine alone. Dilysine is not itself inhibitory, for it does not alter the growth of strain M-123 on media supplemented with glycine. This feature of dipeptide transport is also evidenced by the data presented in Fig. 8, in which dilysine displayed quite definite ability to overcome triornithine inhibition.

**Discussion**

It was previously suggested (3) that, for the transport of the relatively small number of biologically significant amino acids, it is feasible for the cell to possess a number of transport systems with rather high specificities for either individual amino acids or structurally similar ones. This appears to be the situation that does exist with respect to amino acid transport. However, in the case of oligopeptides, the immense variety of structural possibilities precludes the existence of an equivalent number of highly specific oligopeptide transport systems a priori, therefore, it seems that the ideal way to ensure oligopeptide transport resides in designing a minimum number of systems that possess requirements for only those structural features common to all oligopeptides. In accord with this idea, the results presented in this paper provide further evidence for the suggestion made earlier (2, 3), that there appears to be a transport system in E. coli that exhibits little side chain specificity and is essential to the utilization of a variety of oligopeptides.

Previously it was concluded that lysine and ornithine oligopeptides utilize a common transport mechanism in E. coli (3). In the present studies it has been shown (Fig. 2) that M-123, a glycine auxotroph of E. coli, can use glycine oligopeptides as a source of the amino acid, but a mutant strain M-123.TOR, selected for its resistance to triornithine, simultaneously loses the ability to transport glycine as well as ornithine oligopeptides (Fig. 3). It is inferred that ornithine, lysine, and glycine oligopeptides all share a common transport system or, at least, some component of such a system.

The use of a single transport system by a variety of oligopeptides should give rise to competition for entry amongst those oligopeptides. Such competition has been illustrated in various ways. The ability of lysine peptides to curtail the entry of an ornithine peptide is shown in Fig. 8. However, in addition to the proposed competitive exclusion of triornithine, there exist two other mechanisms by which lysine oligopeptides could conceivably overcome the inhibitory effects of triornithine, (a) by the induction of peptidase activity to cleave triornithine, or (b) by protecting the intracellular site sensitive to triornithine. The first point is invalidated by the observation that the level of peptidase activity in strain M-20-26 growing on trilysine is identical with that for cells growing on lysine. In fact, it appears that the enzymatic activity that cleaves trilysine is probably also responsible for the cleavage of triornithine. When trilysine is used as substrate the specific activity of point, there is evidence to suggest that a number of tripeptides of similar structure to triornithine can produce inhibitory effects. Their relative inhibitory efficiencies appear to be related to the potential level of free oligopeptide in the internal pool. In fact, trilysine itself is inhibitory to certain strains of Salmonella typhimurium. Thus, in E. coli trilysine would be expected only to produce inhibitory effects analogous to those of triornithine itself and would not be expected to protect against the tripeptide. The fact that trilysine is not itself inhibitory to E. coli is presumably related to the cells’ high trilysine-splitting activity (3), which precludes an appreciable internal concentration of the free tripeptide.

In the present studies, the large disparity between the affinities for the transport system of the lysine oligopeptides on the one hand and glycine and tyrosine oligopeptides on the other has allowed a detailed study of competitive uptake effects amongst these diverse oligopeptides. However, in an assessment of competitive effects that is dependent upon measurements of growth (the over-all result of many processes of which entry is one only), the situation is complicated by the possibility that competition may occur at sites other than those located at the transport system. In the present case, with the use of auxotrophic strains, the possibility existed that competition could occur at the level of intracellular peptidase activity. For the following reasons this possibility is not considered responsible for the competitive effects described. (a) With the use of crude extracts of E. coli it was shown that the cleavage of triglycine is not inhibited by trilysine at a concentration of trilysine relative to triglycine that is 10 times that at which inhibition of growth of strain M-123 is observed. (b) The enzymatic activity responsible for the cleavage of trilysine has been isolated from E. coli and partially purified. This activity is devoid of peptidase activity toward glycine oligopeptides. It therefore seems unlikely that competitive effects between lysine, glycine, and tyrosine oligopeptides should demonstrate predictable quantitative interrelations if this competition depends upon the inhibition of different peptidases. (c) As discussed above, the most satisfactory explanation of the fact that in the presence of lysine oligopeptides triornithine no longer exerts a bactericidal effect on wild type E. coli is that triornithine is prevented from entering the cells at a rate sufficient to build up a toxic intracellular concentration. The growth of wild type that is observed is not dependent upon the products of peptidase activity. An explanation that invokes peptidases would require not an inhibition of peptidase activity but rather an induction of triornithine-splitting activity by lysine oligopeptides. (d) By competition, the growth rate of strain M-123 could be reduced only to that

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2 In fact, it appears that the enzymatic activity that cleaves trilysine is probably also responsible for the cleavage of triornithine. When trilysine is used as substrate the specific activity of crude extracts is 100 times that found with triornithine as substrate (3). This ratio is preserved throughout the 600-fold purification procedure based upon trilysine-splitting ability (J. Kessler and J. W. Payne, unpublished observations).

3 This possibility is based upon the following observation (J. Kessler, unpublished). When trilysine, triornithine, and mixed tripeptides that contain ornithine and lysine residues are placed in order of increasing growth inhibitory properties, the sequence is identical with that observed when the tripeptides are placed in order of decreasing rate of cleavage by a partially purified peptidase. This peptidase was purified with respect to trilysine cleavage but appears to be responsible for splitting ornithine peptides also (see Footnote 3).

4 J. W. Payne, unpublished observations.

5 B. Furie and J. Kessler, unpublished observations.
observed with the transport-deficient mutant M-123.TOR and no further.

The finding that penta- and hexaglycine use the same transport mechanism as do the lower oligopeptides further strengthens the suggestion made earlier (2, 3) that the failure of pentalysine to enter E. coli cannot be explained by the transport system being unable to accommodate oligopeptides containing more than 4 amino acid residues. Nevertheless, increasing chain length in the glycine series is not without effect, for it was found that relatively higher molar concentrations of the larger oligopeptides are required to achieve a maximum rate of growth. This observation, together with the finding that, when strain M-123 is grown in the presence of equimolar concentrations of triglycine or tetraglycine, less trilysine is required to inhibit growth upon tetra- than upon triglycine, perhaps indicates that the affinity of a glycine oligopeptide for the transport system decreases as the chain length of the peptides increases.

The present work illustrates that in certain cases oligopeptides may enter E. coli by a process that is not susceptible to competition by other oligopeptides (Fig. 10). Furthermore, this mode of entry occurs with a TOR mutant and is therefore believed to be distinct from entry mediated by the general oligopeptide transport system delineated here.

It has been consistently observed with all transport-deficient TOR mutants that, although the ability to transport oligopeptides has been lost, the ability to transport dipeptides persists. Furthermore, it was reported in an earlier publication that a lysine dipeptide lacking a terminal carboxyl group could enter strain M-26-26 but could not enter M-26-26.TOR (3). As it was shown that the oligopeptide transport system did not require the presence of the carboxyl group, it was concluded from the above result that the dipeptide without the carboxyl group used the oligopeptide system to enter E. coli and that it was unable to enter the TOR mutant because the dipeptide transport system possesses the additional requirement for the presence of the terminal carboxyl group. In the present studies this inferred ability of dipeptides to use the oligopeptide transport system has been corroborated by the observation that dilysine can inhibit the growth of strain M-123 on triglycine (Fig. 12) and can also alleviate the inhibitory effects of triornithine (Fig. 8). However, as indicated by the characteristics of the TOR mutation and substantiated here by the finding that trilysine cannot prevent a dipeptide from entering the cell, oligopeptides are not able to use the dipeptide transport system or systems. This is exactly the result one would expect on the basis of the previous findings concerning carboxyl group requirements, for tripeptides and higher peptides can be considered as dipeptides with substituted terminal carboxyl groups and therefore should not be able to use the dipeptide transport system that appears to require specifically an unsubstituted carboxyl group.

The natural habitat of E. coli is the peptide-rich lumen of the gut, and it would therefore appear advantageous for this organism to possess a mechanism for concentrating peptides. In attempts to assess how general is the occurrence of such a mechanism, it has been found that a lysine auxotroph of Staphylococcus aureus can use homologous and heterologous lysine oligopeptides as sources of the required amino acid and that the requirements of two strains of Bacillus megaterium for lysine and ornithine can be met by the appropriate oligopeptides. Further work will be required to establish whether identical transport systems are operative in these diverse organisms.

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