0021-9193/78/0133-0165$02.00/0
JOURNAL OF BACTERIOLOGY, Jan. 1978, p. 165-171

Vol. 133, No. 1
Printed in U.S.A.

Peptide Utilization in *Pseudomonas aeruginosa*: Evidence for Membrane-Associated Peptidase

ROBERT V. MILLER* AND JEFFREY M. BECKER

Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37916

Received for publication 14 July 1977

A methionine auxotroph of *Pseudomonas aeruginosa* grew on methionine-containing peptides as a source of the required amino acid. Amino-terminus-blocked peptides would not serve as growth substrates, despite the fact that peptidases active on these blocked peptides were readily detectable in cell extracts. No evidence was found for extracellular enzymes capable of degrading the oligopeptides investigated. The degradative enzymes were not found in the periplasmic space of the cellular envelope. A high proportion of cellular peptidase activity was associated with the particulate (membrane) fraction of the cell lysate.

The introduction of impermeant molecules into bacterial cells by covalent attachment to transportable peptides has been achieved (1, 11). This development has great potential for the study of amino acid analog metabolism and the design of antimicrobial agents (19), since normally nonpenetrating moieties can be placed within the cell. To utilize this technique with specific microorganisms, it is necessary to identify the abilities and limitations of these specific microbial cells to both transport and utilize oligopeptides. Studies on a variety of organisms, including bacteria (2, 28, 33), fungi (19, 26, 36), and mammals (22), have indicated that some similarities exist between organisms in the general characteristics of peptide transport. However, other evidence indicates that some parameters of peptide transport vary from species to species (19).

We have been interested in studying *Pseudomonas aeruginosa*, an ubiquitous microorganism that is an opportunistic pathogen. Infection by *P. aeruginosa* reduces the probability of a positive prognosis for patients traumatized by burns or undergoing immunosuppressive therapy as well as for those individuals suffering from the genetic disorder cystic fibrosis. Recently, peptide utilization in *Pseudomonas putida* and *Pseudomonas maltophilia* was examined (7, 8); similar data for *P. aeruginosa* have not been reported. Our investigation demonstrates several differences in peptide utilization by *P. aeruginosa* as compared with other gram-negative bacteria such as *P. putida*, *Escherichia coli*, and *Salmonella typhimurium*.

MATERIALS AND METHODS

Bacterial strains and cultivation conditions. RM46, a methionine auxotroph of *P. aeruginosa* strain PAO, was used throughout this study. The lesion in methionine biosynthesis in this strain is in the *metIV* transduction group, as described by Calhoun and Feary (6).

Stock cultures were maintained on Luria complete broth composed of 10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, and 0.1 g of NaOH in 1 liter of distilled water. Experimental cultures were grown overnight at 37°C with aeration in pseudomonas minimal medium (PMM), which contained potassium phosphate buffer (60 mM, pH 7.2), sodium citrate (1.7 mM), $MgSO_4 \cdot 7H_2O$ (0.4 mM), $(NH_4)_2SO_4$ (7.5 mM), and 0.4% glucose or in a minimal medium containing tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (0.12 M, pH 7.2), KCl (2 mM), $MgSO_4 \cdot 7H_2O$ (1.6 mM), $FeCl_3$ (9 μM), $(NH_4)_2SO_4$ (8 mM), and 0.4% glucose (PTM medium). Filter-sterilized methionine or methionine-containing peptides were added as indicated in the text. Cultures were inoculated from starter cultures grown overnight at 37°C in PMM containing D,L-methionine (50 μg/ml).

Chemicals and enzymes. L-Methionine (L-Met), D-Met, and Met-Met were purchased from Sigma Chemical Co., St. Louis, Mo. $(Met)_3$, Met-Gly-Gly, Gly-Met-Gly, and Met-Gly-Met-Met were purchased from Schwarz/Mann, Orangeburg, N.Y. All amino acid residues of all peptides were in the L-configuration. All other peptides, including radioactive trimethionine (4), were the kind gift of F. Naider, Department of Chemistry, College of Staten Island of the City University of New York. All peptides were judged homogeneous after analysis by high-voltage electrophoresis and thin-layer chromatography. Lysozyme, deoxyribonuclease A, and alkaline phosphatase were from Worthington Biochemicals Corp., Freehold, N.J. Phosphatase substrate was purchased from Sigma.

Cell lysis procedures. Two methods were used to lyse cells. Method I was used for cells grown in PMM medium and was a modification of the method of Miller and Clark (24). Cells from late log phase were harvested, washed in a 0.85% solution of NaCl, and resuspended in 10% sucrose in 50 mM Tris-hydrochloride buffer (pH 7.5, 0.5 g [wet weight] of cells/ml).

The cell suspension was frozen quickly in an ethanol–dry-ice bath and allowed to thaw at room temperature. For each milliliter of cell suspension, 0.1 ml of 1 M NaCl was added to the suspension. The suspension was incubated at 0°C until lysis had occurred (30 to 45 min). Magnesium sulfate was added to the cell lysate to a final concentration of 10 mM. Ten microliters of a deoxyribonuclease I solution (10 mg/ml in 50 mM Tris-hydrochloride, pH 8.0) was added per milliliter of cell lysate to digest DNA and reduce the viscosity of the solution. The cell lysate was centrifuged at 20,000 × *g* for 60 min at 4°C. The supernatant (soluble) fraction was separated from the particulate (membrane) fraction by decantation and each fraction was dialyzed separately against 50 mM Tris-hydrochloride (pH 8.0) overnight at 4°C. Fractions were stored frozen until needed.

The second method (method II) of cell lysis was used for cells grown in PTM medium. In this lysis procedure, cells were harvested and washed in a 0.85% solution of NaCl and resuspended in 10% sucrose in 50 mM Tris-hydrochloride buffer (pH 7.5, 0.5 g [wet weight] of cells/ml). For each milliliter of cell suspension, 0.1 ml of lysozyme (2 mg/ml in Tris-hydrochloride, pH 8.0), 0.1 ml of 1 M NaCl, and 10 μl of deoxyribonuclease I (10 mg/ml in 50 mM Tris-hydrochloride, pH 8.0) were added to the suspension. The cell suspension was sonically treated using four 10-s pulses of a Branson Sonifier (model WI85) set at 3.5. The cell lysate was centrifuged for 60 min at 20,000 × *g* and 4°C. The soluble and membrane fractions were separated and dialyzed as above. In both methods, cell viability was reduced greater than 99%. No detectable activity (<0.1 unit) of the cytoplasmic deoxyribonucleases *Pae*ExoI and *Pae*ExoV (24) was present in the membrane fractions.

Peptidase assay and electrophoresis. A portion of the cellular fraction to be assayed (usually 100 μl) was incubated at 37°C with an equal volume of peptide solution (1 mg/ml in distilled water). At various time intervals, 50-μl portions of the reaction mixture were removed and applied to Whatman 3MM paper for electrophoresis. Electrophoresis was carried out in a model LT-36 electrophoresis tank, E.C. 123 coolant, and an HV-5000 power supply (Savant Instruments). Pyridine acetate buffer (pH 3.5) prepared from glacial acetic acid-pyridine-water (10:1:89, vol/vol/vol) was used. The electrophoretogram was run at 50 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 a well-ventilated oven.

Coupled-enzyme assay for peptidase activity. A rapid quantitative analysis of enzyme activity was carried out according to a modification of the procedure of Fujita et al. (12). Breakdown of $(Met)_3$ was followed by determining the amount of free methionine produced. The free amino acids were oxidized to keto acids by the action of L-amino acid oxidase with the liberation of hydrogen peroxide. The H_2O_2, in turn, was degraded by horseradish peroxidase. In this reaction molecular oxygen was produced, resulting in oxidation of *o*-dianisidine to a colored form, which was measured spectrophotometrically.

To carry out this procedure, a reaction mixture containing 0.8 ml of trimethionine solution (0.4 mg/ml in 50 mM Tris, pH 8.0), 0.1 ml of an appropriate dilution of the peptidase source, and 0.1 ml of 50 mM Tris (pH 8.0) was prepared. At 0, 10, and 20 min, 0.1 ml of the reaction mixture was removed and brought to 100°C for 3 min to stop the enzymatic reaction. Each boiled fraction was cooled and added to 2.0 ml of the coupled enzyme reagent (L-amino acid oxidase [120 μg, from *Crotalus adamanteus* venom; Sigma], horseradish peroxidase [20 μg; Sigma], and *o*-dianisidine [0.16 mM; Sigma] in Tris-hydrochloride buffer [50 mM, pH 8.0]) and incubated at room temperature for 1 h. The amount of free methionine in the sample was determined by measuring the change in absorbancy at 440 nm, using a Beckman Acta CIII spectrophotometer. L-Methionine (Eastman Chemical Corp.) was used as a standard.

Release of periplasmic localized proteins. Treatment of cell suspensions to release proteins present in the periplasmic component was carried out by a modification of the method of Cheng et al. (9). After growth of cultures in PTM to late log phase, cells were harvested and washed in a 0.85% solution of NaCl. The cells were then "shocked" by incubation for 40 min at 21°C in 0.2 M $MgCl_2 \cdot 7H_2O$ in 0.1 M Tris-hydrochloride (pH 8.4; magnesium-Tris buffer). The cell suspension was then centrifuged for 5 min at 11,000 × *g* and 4°C. The supernatant fluid (shockate) was decanted and dialyzed against Tris-hydrochloride (50 mM, pH 8.0) overnight at 4°C. The shocked cells were resuspended in 10% sucrose in 50 mM Tris-hydrochloride (pH 7.5) and lysed by method II as described above. The fractions (membrane and soluble) produced were dialyzed overnight as described above.

Other methods. Alkaline phosphatase was assayed by the method of Torriani (34). The reaction was followed at 37 C at 410 nm in a Beckman Acta C III recording spectrophotometer. One unit of enzyme is the amount needed to produce a change of optical density of 1.0 per minute at 410 nm. Protein determinations were by the method of Lowry et al. (21), using bovine serum albumin as a standard. Scintillation counting of electrophoretograms was in a Packard Tri-Carb scintillation counter with 10 ml of scintillation fluid {2,5-diphenyloxazole (4.0 g/liter) and 1,4-bis-[2]-(5-phenyloxazolyl)benzene (0.5 g/liter) in toluene}. Electrophoretograms were prepared for counting by cutting a 4-cm-wide strip centered on the point of application of the sample and parallel with the direction of migration. This strip was cut into sections 1 cm in length, starting 2 cm before the origin and proceeding in the direction of migration of the peptides. Each section was counted separately in the above scintillation fluid. Marker amino acids and peptides were co-electrophoresed as standards.

RESULTS

Growth on methionine-containing peptides and derivatives. The ability of peptides to supply an amino acid auxotroph with a required amino acid has been used successfully to ascertain the ability of various cells to transport and degrade peptides (7, 13, 19, 26, 36). We used

this rationale for an initial survey of the utilization of methionine-containing peptides by strain RM46, a methionine auxotroph of *P. aeruginosa*. The growth response was determined in 10 ml of PMM containing methionine (10 μg/ml) or peptides at a concentration to give an equal number of mole-equivalents of methionine per milliliter of medium. This methionine concentration allowed maximum growth rate of the organism and was the limiting factor to final cell yield. The data in Fig. 1 and Table 1 summarize the growth response of this strain on methionine and a series of methionine-containing peptides. Growth was absolutely dependent upon the presence of the amino acid methionine. In addition to methionine, several oligopeptides, including di-, tri-, tetra-, and pentapeptides, containing methionine served as a source of this required amino acid. In all cases, the growth response (length of lag, growth rate, cell yield) in the presence of these peptides was not significantly different than when the free amino acid was supplied directly. *P. aeruginosa* was unable to utilize several peptides in which the amino terminus was derivatized. Acetylated peptides [i.e., Ac-$(Met)_3$] could not serve as a source for methionine nor could tripeptides containing the larger group *tert*-butyloxycarbonyl [i.e., Boc-$(Met)_3$]. Similar peptides with nonderivatized amino termini served as growth substrates. In contrast, when the carboxy terminus of trimethionine was blocked by esterfication [$(Met)_3$OMe, trimethionine methyl ester], the peptide was still utilizable as a source for methionine.

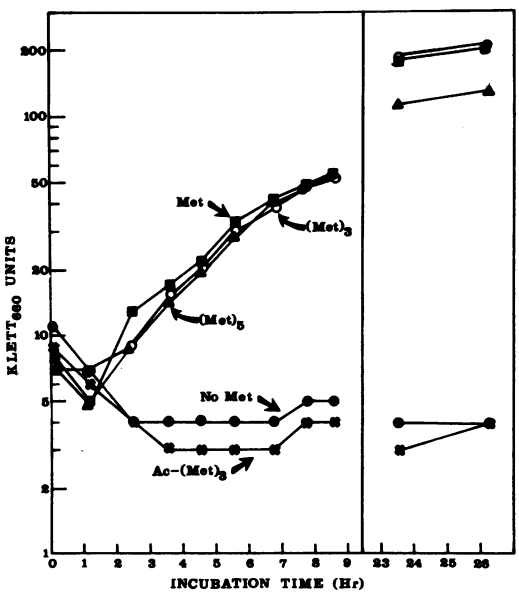

FIG. 1. *Growth response of P. aeruginosa strain RM46 to various methionine-containing peptides. PMM was supplemented with* L-*methionine* (■), *(Met)*$_3$ (○), *(Met)*$_5$ (▲), *Ac-(Met)*$_3$ (×), *or no methionine* (●). *All peptides were supplemented so that the final concentration of methionine was 10 μg/ml.*

TABLE 1. *Growth response of P. aeruginosa strain RM46 to methionine-containing peptides*[a]

Methionine source[b]	Growth response	
	Generation time (h/generation)	Klett units (660 nm) at 24 h
No Met	NG[c]	4
Met	2.6	180
Dipeptides		
Met-Met	2.4	210
Gly-Met	2.1	220
Met-Gly	2.1	210
Tripeptides		
$(Met)_3$	2.6	195
Met-Gly-Gly	2.4	240
Gly-Met-Gly	2.6	160
Gly-Gly-Met	2.8	170
Other oligopeptides		
Met-Gly-Met-Met	2.1	200
Met-Met-Gly-Met	2.1	100
Met-Met-Met-Gly	2.3	130
$(Met)_5$	2.2	130
Blocked peptides		
Ac-$(Met)_3$	NG	3
Boc-$(Met)_3$	NG	13
Ac-Met-Gly-Met	NG	2
$(Met)_3$-OMe	2.5	130

[a] All peptides were supplemented to the same level of required amino acid (10 μg/ml).
[b] All residues are in the L-configuration.
[c] NG, No growth on this methionine source.

Peptidase activity in cell lysates. The inability of *P. aeruginosa* to utilize peptides that are blocked at the amino terminus could result from one of two reasons: either the cell does not contain peptidase capable of hydrolyzing the peptide or the cell is unable to transport the peptide into the cell. One way to distinguish between these two possibilities is to determine the presence of an intracellular peptidase capable of degrading the nonutilizable peptide. If such an enzyme is present, one can infer that the disallowed step in the utilization of the peptide is its impermeability to the cellular membrane. Cells were grown in PMM medium containing methionine (10 μg/ml) and harvested, and cell lysates were prepared by the lysozyme, freeze-thaw method described in Materials and Methods. The supernatant fraction was tested for peptidase activity by incubation with $(Met)_2$, $(Met)_3$, $(Met)_5$, $(Met)_3$-OMe, Ac-$(Met)_3$, and Boc-$(Met)_3$ and analysis by high-voltage electrophoresis. All oligopeptides tested were hydrolyz-

able by the cell extract. This included both Ac-$(Met)_3$ and Boc-$(Met)_3$, two nonutilizable, amino-blocked peptides. From these results, it would appear that the inability of *P. aeruginosa* to utilize peptides with derivatized amino termini is due to the inability of the cell to take up the peptide.

Localization of peptidase activity. Several experiments were carried out to determine the location of the peptidase(s) responsible for the degradation of $(Met)_3$ and similar peptides (see Table 1). In our first experiments, we asked if detectable amounts of peptidase activity could be found in culture medium in which *P. aeruginosa* had been grown. *P. aeruginosa* strain RM46 was inoculated into 100 ml of PMM containing $(Met)_3$ at 6.7×10^{-5} M and incubated until late log phase (100 Klett units at 660 nm). The cells were harvested, and the culture medium (spent medium) was filtered through a 0.45-μm cellulose nitrate filter. The cells were lysed, and particulate and supernatant fractions were prepared as described in Materials and Methods. The spent medium and cellular fractions were dialyzed, and peptidase activity was determined by the electrophoresis assay except that the reaction mixture contained radioactive trimethionine (specific activity, 1.1 mCi/mmol) labeled with ^{14}C in the carboxy-terminal methionine residue. Samples were taken at 0, 1, 3, and 5 h, and the nanomoles of ^{14}C-labeled $(Met)_2$ liberated was determined for each time point. The results of these assays are depicted in Fig. 2. There was no detectable breakdown of $(Met)_3$ by the spent medium during this time period, and, in fact, when reaction mixtures were allowed to incubate for as long as 24 h there was still no detectable ^{14}C-labeled $(Met)_2$ or ^{14}C-labeled Met produced. In other experiments, any macromolecules present in the spent medium were concentrated 100-fold, using an Amicon ultrafiltration unit (Amicon Corp., Lexington, Mass.). There was no detectable peptidase activity in these concentrated samples. From these experiments, it would appear that extracellular peptidase active on $(Met)_3$ is not produced by *P. aeruginosa*.

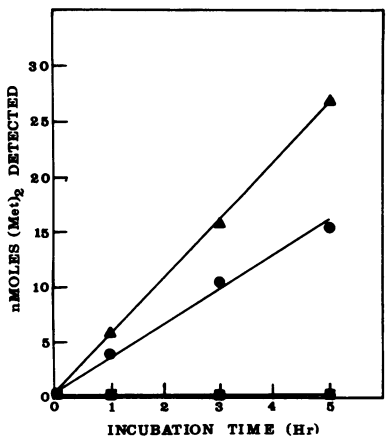

FIG. 2. *Peptidase activity of cellular fractions on ^{14}C-labeled $(Met)_3$. Reaction conditions and analysis of radioactivity were as described in Materials and Methods. Cell supernatant fluid (▲), membrane fraction (●), and spent medium (■) were tested.*

Cellularly associated peptidase activity may be external to the inner membrane and located in the periplasmic space. In this case, degradation of the peptide may take place followed by uptake of the free amino acid. Cheng et al. (9) found that enzymes associated with the periplasmic space, for example, alkaline phosphatase, are released by "shocking" the cell with 0.2 M Mg^{2+} at pH 8.4. We used a modification of this method to determine whether or not the peptidases associated with *P. aeruginosa* were shockable from the periplasmic region of the cell membrane. For these experiments, cells were grown in PTM supplemented with 10 μg of methionine per ml. After growth, the cells were washed and shocked in magnesium buffer. After collection of the shockate, the cells were lysed by method II and the soluble and particulate fractions were collected. The growth response of cells grown in PTM to various methionine-containing peptides, especially $(Met)_3$ and Ac-$(Met)_3$, was the same as cells grown in PMM (Fig. 1, Table 1). The magnesium wash, supernatant, and particulate fractions were tested for alkaline phosphatase and peptidase activity (Table 2). Even when over 75% of the alkaline phosphatase activity was removed from the periplasmic space, there was no release of peptidase activity. In additional experiments, no peptidase activity was detectable when the amount of shock fluid in the reaction mixture was increased 10-fold.

The experiments described in the last section were surprising to us in that a high percentage of the peptidase activity was found associated with the particulate fraction (Fig. 2). In different preparations the fraction of peptidase activity associated with the particulate fraction ranged from 35 to 75% of the total specific activity. Since the activity exhibited in this fraction might be due to fortuitous association, the particulate fraction was repeatedly washed by resuspension in 50 mM Tris-hydrochloride (pH 8.0) buffer and centrifugation at 20,000 × *g* for 60 min. This process was repeated four times. During each round, a sample was retained from the supernatant wash fluid and the particulate fraction. Each of these samples was assayed for

peptidase activity using the coupled enzyme assay of Fujita et al. (12). The degradation of $(Met)_3$ was analyzed, and the protein content of each sample was determined. The peptidase activity per milligram of protein was calculated (Table 3). By the third and fourth wash cycles, peptidase activity was not detectable in the supernatant wash fluid, whereas a significant amount (33% of the total activity) was still bound to the membrane fraction. In additional experiments, it was found that peptidase activity capable of degrading Ac-$(Met)_3$ was also found in significant amounts in the membrane fraction even after four washings.

DISCUSSION

In this paper we have demonstrated that peptides can be utilized in place of the required amino acid by a methionine auxotroph of *P. aeruginosa*. Some features of this peptide utilization are similar to those demonstrated for other gram-negative bacteria (2, 7, 28). For *E. coli* (2), the sequence of amino acid residues in the peptides is not important in determining its ability to serve as an amino acid source. Similarly, Met-Gly-Gly, Gly-Met-Gly, and Gly-Gly-Met serve equally well as a source of methionine for *P. aeruginosa*. In addition, a free amino terminus is necessary for methionine-containing peptide utilization, whereas a free carboxyl terminus is not important for either *P. aeruginosa* or *E. coli* (3, 25). On the other hand, one important difference in peptide utilization between *P. aeruginosa* and the other gram-negative bacteria examined is notable. All the evidence (2, 28) reported to date indicates that peptides are transported intact into bacteria and are then hydrolyzed by intracellular peptidases, whereas the data reported in this study indicate that a different mode of peptide utilization may exist in *P. aeruginosa*.

P. aeruginosa produces many extracellular products, including proteases (16), which have been implicated in the pathogenicity of the organism (16, 19, 20). For our studies on peptide utilization, therefore, it was especially important to determine whether peptidases were released into the environment by this bacterium. The existence of extracellular peptidases would negate the use of peptide-mediated illicit transport (1) of potentially toxic materials as a mechanism for the control of this organism. Even though no extracellular peptidase was detected in the culture medium in which *P. aeruginosa* was grown, a significant amount of the peptidase activity towards both $(Met)_3$ and Ac-$(Met)_3$ could not be washed from the particulate fraction of a cellular lysate.

There have been only a few reports of such membrane-bound peptidases in bacteria. Lazdunski and co-workers (18) identified a periplasmic aminoendopeptidase in *E. coli*. However, although the purified enzyme cleaved various amino acid-*p*-nitroanilides and amino acid-β-naphthylamides, the enzyme had no activity to the small peptides Ala-Gly-Gly, Ala-Val, Ala-Pro, or Ala-Ser. DD-Carboxypeptidases have been found associated with the particulate fractions of cell lysates of several bacteria (5, 14, 30). The predominance of evidence from the laboratories of Simmonds and Toye (32) and others (2, 23, 27, 28) indicates that peptidases capable of hydrolyzing peptide bonds between two L-amino acids are strictly intracellular in *E. coli*. Peptidase activity has been found associated with the membrane and cytoplasmic fractions of *Mycoplasma laidlawii* (10, 29). The

TABLE 2. *Release of peptidase and alkaline phosphatase activity from the periplasmic space by osmotic shock*

Sample	Alkaline phosphatase activity[a] (units/mg of protein)	$(Met)_3$-directed peptidase activity[b]
Culture medium	<0.01	−
0.2 M $MgCl_2$ wash	0.36	−
Supernatant fraction of cell lysate	0.03	+
Membrane fraction	0.08	+

[a] Assays were as described in Materials and Methods. One unit of activity is defined by Torriani (34) as the amount of enzyme necessary to produce a change of 1 optical density unit at 410 nm in 1 min.

[b] Peptidase assays were for 4 h at 37°C, using the electrophoresis assay conditions. −, No detectable $(Met)_3$ degradation; +, significant degradation of $(Met)_3$ with liberation of $(Met)_2$ and Met. Often there was complete degradation of $(Met)_3$ to free methionine. All reaction mixes contained the same level of protein (ca. 0.1 mg).

TABLE 3. *Peptidase activity associated with the particulate fraction after repeated washing*

Procedure	Supernatant fluid		Particulate fraction	
	Total protein (mg)	Sp act[a]	Total protein (mg)	Sp act[a]
Cell lysate	54	2.8	62	17.7
Wash I	22	5.8	47	12.2
Wash II	7	7.2	30	7.1
Wash III	3	<0.1	25	8.3
Wash IV	2	<0.1	19	6.4

[a] Activity is expressed in micromoles of methionine liberated at 37°C in 20 min per milligram of protein. Activity was assayed by the coupled enzyme assay described in Materials and Methods.

enzyme described in *Mycoplasma* appeared to be located on the outside of the membrane. The evidence we have accumulated indicates that this is not the case for the *P. aeruginosa* peptidase activity. The fact that no peptidase activity could be shocked from the periplasmic space under conditions that removed the majority of the periplasmic-associated alkaline phosphatase indicates that peptidases are not present in the periplasmic region. In addition, the nonutilization of Ac-$(Met)_3$ as a source of methionine, even though significant amounts of peptidase able to degrade Ac-$(Met)_3$ were found in the membrane fraction, argues that at least this peptidase is not accessible in vivo to blocked tripeptides.

In recent reviews, Payne (28) and Barak and Gilvarg (2) proposed several models of peptide utilization in bacteria. Genetic and kinetic evidence in *E. coli* (2, 17, 28) and *S. typhimurium* (15) support a model of transport followed by degradation of the peptide, whereas another proposed model is based on an intimate association of transport and degradation of peptides. This close coupling model of transport and hydrolysis has been proposed for intestinal peptide transport (35) and peptide transport in eucaryotic tissue containing γ-glutamyl transpeptidase (31). *P. aeruginosa* may provide a system for an analysis of a model of the interaction of transport and degradation in the utilization of peptides by microorganisms.

ACKNOWLEDGMENTS

We thank Cheryl Whitlock, Sam Cowen, Donald Lewis, and Melanie Haas for their efficient and professional help in carrying out certain of the experiments reported here.

This work was supported by Public Health Service grant GM 22087 from the National Institute of General Medical Sciences to J. M. B. and a grant from the National Cystic Fibrosis Research Foundation to R.V.M. J.M.B. is a recipient of Research Career Development Award GM-00094 from the Public Health Service.

LITERATURE CITED

1. **Ames, B. N., G. F. Ames, J. D. Young, D. Tsuchiya, and J. Lecocq.** 1973. Illicit transport: the oligopeptide permease. Proc. Natl. Acad. Sci. U.S.A. **70**:456–458.
2. **Barak, Z., and C. Gilvarg.** 1975. Peptide transport, p. 167–218. *In* H. Eisenberg, E. Katchalski-Katzir, and L. A. Manson (ed.), Biomembranes, vol. 7. Plenum Press, New York.
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. **Becker, J. M., and F. Naider.** 1977. Peptide transport in yeast: uptake of radioactive trimethionine in *Saccharomyces cerevisiae*. Arch. Biochem. Biophys. **178**:245–255.
5. **Blumberg, P. M., and J. L. Strominger.** 1971. Inactivation of D-alanine carboxypeptidase by cephalosporins is not lethal to *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **68**:2814–2817.
6. **Calhoun, D. H., and T. W. Feary.** 1969. Transductional analysis of *Pseudomonas aeruginosa* methionineless auxotrophs. J. Bacteriol. **97**:210–216.
7. **Cascieri, T., Jr., and M. F. Mallette.** 1976. Peptide utilization by *Pseudomonas putida* and *Pseudomonas maltophilia*. J. Gen. Microbiol. **92**:283–295.
8. **Cascieri, T., Jr., and M. F. Mallette.** 1976. Intracellular peptide hydrolysis by *Pseudomonas putida* and *Pseudomonas maltophilia*. J. Gen. Microbiol. **92**:296–303.
9. **Cheng, K. J., J. M. Ingram, and J. W. Costerton.** 1970. Release of alkaline phosphatase from cells of *Pseudomonas aeruginosa* by manipulation of cation concentration and of pH. J. Bacteriol. **104**:748–753.
10. **Choules, G. L., and W. R. Gray.** 1971. Peptidase activity in the membranes of *Mycoplasma laidlawii*. Biochem. Biophys. Res. Commun. **45**:849–855.
11. **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.
12. **Fujita, M., D. S. Parsons, and F. Wojnorowska.** 1972. Oligopeptidases of brush border membranes of rat small intestinal mucosal cells. J. Physiol. **227**:377–394.
13. **Gilvarg, C., and E. Katchalski.** 1965. Peptide utilization in *Escherichia coli*. J. Biol. Chem. **240**:3093–3098.
14. **Izaki, K., and J. L. Strominger.** 1968. Purification and properties of two D-alanine carboxypeptidases from *E. coli*. J. Biol. Chem. **243**:3193–3201.
15. **Jackson, M. B., J. M. Becker, A. Steinfeld, and F. Naider.** 1976. Oligopeptide transport in proline peptidase mutants of *Salmonella typhimurium*. J. Biol. Chem. **251**:5300–5309.
16. **Johnson, G. G., J. M. Morris, and R. S. Beck.** 1967. The extracellular protease from *Pseudomonas aeruginosa* exhibits elastase activity. Can. J. Microbiol. **13**:711–719.
17. **Kessel, D., and M. Lubin.** 1963. On the distinction between peptidase activity and peptide transport. Biochim. Biophys. Acta **71**:656–663.
18. **Lazdunski, C., J. Busuttil, and A. Lazdunski.** 1975. Purification and properties of a periplasmic amino endopeptidase from *Escherichia coli*. Eur. J. Biochem. **60**:363–369.
19. **Lichliter, W. D., F. Naider, and J. M. Becker.** 1976. A basis for the design of anti-candidal agents from studies of peptide utilization in *Candida albicans*. Antimicrob. Agents Chemother. **10**:483–490.
20. **Liu, P. V.** 1974. Extracellular toxins of *Pseudomonas aeruginosa*. J. Infect. Dis. **130**(Suppl.):S94–S99.
21. **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.
22. **Mathews, D. M.** 1975. Intestinal absorption of peptides. Physiol. Rev. **55**:537–608.
23. **Miller, C. G.** 1975. Peptidases and proteases of *Escherichia coli* and *Salmonella typhimurium*. Annu. Rev. Microbiol. **29**:485–504.
24. **Miller, R. V., and A. J. Clark.** 1976. Purification and properties of two deoxyribonucleases of *Pseudomonas aeruginosa* strain PAO. J. Bacteriol. **127**:794–802.
25. **Naider, F., and J. M. Becker.** 1975. Multiplicity of oligopeptide transport systems in *Escherichia coli*. J. Bacteriol. **122**:1208–1215.
26. **Naider, F., J. M. Becker, and E. Katchalski.** 1974. Utilization of methionine-containing peptides and their derivations by a methionine requiring auxotroph of *Saccharomyces cerevisiae*. J. Biol. Chem. **249**:9–20.
27. **Neu, H. C., and L. A. Heppel.** 1965. The release of enzymes from *Escherichia coli* by osmotic shock and during the formation of spheroplasts. J. Biol. Chem. **240**:3685–3692.
28. **Payne, J. W.** 1976. Peptides and microorganisms. Adv. Microbiol. Physiol. **13**:55–113.
29. **Pecht, M., E. Giberman, A. Keysary, J. Yariv, and E. Katchalski.** 1972. Hydrolysis of alanine oligopep-

tides by an enzyme located in the membrane of *Mycoplasma laidlawii*. Biochim. Biophys. Acta **290**:267–277.

30. **Presslitz, J. E., and V. A. Ray.** 1975. DD-Carboxypeptidase and peptidoglycan transpeptidase from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **7**:578–581.
31. **Prusiner, S., C. W. Doak, and G. Kirk.** 1976. A novel mechanism for group translocation: substrate-product neutralization by γ-glutamyl transpeptidase in peptide and amino acid transport. J. Cell Physiol. **89**:853–864.
32. **Simmonds, S., and N. O. Toye.** 1966. Peptidases in spheroplasts of *Escherichia coli* K-12. J. Biol. Chem. **241**:3852–3860.
33. **Sussman, A. J., and C. Gilvarg.** 1970. Peptide transport and metabolism in bacteria. Annu. Rev. Biochem. **40**:397–408.
34. **Torriani, A.** 1968. Alkaline phosphatase of *Escherichia coli*. Methods Enzymol. **12B**:212–218.
35. **Ugolev, A. M., and P. Delaey.** 1973. Membrane digestion. A concept of enzymatic hydrolysis on cell membrane. Biochim. Biophys. Acta **300**:105–128.
36. **Wolfinbarger, L., Jr., and G. A. Marzluf.** 1974. Peptide utilization by amino acid auxotrophs of *Neurospora crassa*. J. Bacteriol. **119**:371–378.