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# Peptide Synthesis in Cell-Free Extracts of *Bacillus brevis* 8185*

R. K. Rao, N. V. Bhagavan,† K. R. Rao, and J. B. Hall

ABSTRACT: The biosynthesis of antibiotic polypeptides by cell-free extracts of *Bacillus brevis* ATCC 8185 is described.

Optimal conditions for tyrocidine biosynthesis were determined. No gramicidin-synthesizing activity was observed, and the only tyrocidine produced in significant amounts was tyrocidine D. It appears that this strain differs in this respect from *B. brevis* ATCC 10068, which produces the same spectrum of polypeptides, but does not appear to synthesize any one of the tyrocidines preferentially under the same conditions.

The recent controversy over the nature of the system that synthesizes gramicidin S has been largely resolved by the agreement of all groups concerned that an enzymatic, nonribosomal mechanism is involved (Yukioka *et al.*, 1965; Berg *et al.*, 1965; Bhagavan *et al.*, 1966; Tomino *et al.*, 1967). It seemed likely that a similar system was involved in the biosynthesis of the antibiotic polypeptides of the tyrocidine and gramicidin families produced by other strains of *Bacillus brevis* and clearly related to gramicidin S in structure and biological activity. A report from Fujikawa *et al.* (1966) suggested that a nonribosomal system was indeed utilized for tyrocidine production. The only other work on cell-free extracts, however, had implicated a ribosomal pathway for this synthesis (Bodley *et al.*, 1964), but in view of the apparently erroneous reports published by this same group of workers on gramicidin S biosynthesis (Hall *et al.*, 1965) and later corrected (Bhagavan *et al.*, 1966), it seemed worthwhile to reexamine the question of tyrocidine biosynthesis, in order to resolve the conflict in the literature.

The present work confirms and extends the findings of Fujikawa *et al.* (1966), that the synthetic system is nonribosomal in nature. The extracts have been characterized in more detail and additional observations on the biosynthesis of tyrocidines and gramicidins were carried out. No technical problems were encountered in the course of this work which could help to explain the earlier erroneous reports, and it seems unlikely at this time that a scientific explanation for these discrepancies will be forthcoming.

As this paper was being prepared for publication, we received a copy of a manuscript from Fujikawa *et al.* (1968) describing their work on the purification and properties of the tyrocidine-synthesizing system. Their preparation appears to be similar in all essentials to the one we describe here, and in addition, the partial purification and fractionation of the active extract is reported.

## Materials and Methods

*Chemical and Enzymes.* Puromycin was obtained from Nutrional Biochemicals Corp. Chloramphenicol was purchased from Parke Davis Co. Gramicidin and tyrocidine were obtained from Mann Chemical Co. Amino acids, PEP,[1] and ATP were supplied by Sigma Chemical Co. CTP, GTP, and UTP were products of Calbiochem. All of the isotopic compounds were obtained from New England Nuclear Corp. and had the following specific activities (millicuries per millimole): L-[U-$^{14}$C]-alanine, 117; L-[U-$^{14}$C]aspartic acid, 167; L-[U-$^{14}$C]-asparagine, 46.9; [1,2-$^{14}$C]ethanolamine, 4.34; [U-$^{14}$C]-glycine, 116; L-[U-$^{14}$C]glutamic acid, 195; L-[U-$^{14}$C]-glutamine, 52.3; DL-[1-$^{14}$C]leucine, 31; L-[U-$^{14}$C]leucine, 251.4; DL-[5-$^{14}$C]ornithine, 11.0; DL-[3-$^{14}$C]-phenylalanine, 4.46; DL-[5-$^{14}$C]proline, 5; L-[U-$^{14}$C]serine, 120; DL-[3-$^{14}$C]tryptophan 1.93; DL-[1-$^{14}$C]tyrosine, 2.4; DL-[1-$^{14}$C]valine, 18.5; and uniformly labeled

* From the Department of Biochemistry and Biophysics, University of Hawaii, Honolulu, Hawaii. *Received March 25, 1968.* This research was supported by a grant from the National Institutes of Health (GM 13312).

† Presently on the faculty of Hawaii Loa College, Honolulu, Hawaii.

[1] Abbreviation used that is not listed in *Biochemistry 5*, 1445 (1966), is: PEP, phosphoenolpyruvate.

amino acid mixture (NEC-445, about 1 mCi/mg). Crystalline pancreatic deoxyribonuclease (EC 3.1.4.5) and ribonuclease (EC 2.7.7.16) were products of Worthington Biochemical Corp. Crystalline pyruvic kinase (EC 2.7.1.40) was a product of Calbiochem. Sephadex G-25 was obtained from Pharmacia Fine Chemicals, Inc.

*Bacterial Culture Conditions. B. brevis* strain ATCC 8185 was grown at 37° in stationary culture in a tryptone–mineral salts medium enriched with 0.5% yeast extract and 1% glucose (Okuda *et al.*, 1963). The density of the culture was measured at 650 mμ in the Beckman DU spectrophotometer. Optimal results were obtained with cells harvested between absorbancies of 1.19 and 1.33. *B. brevis* ATCC 10068 was grown as described by Mach *et al.* (1963), with the exception that 2% glucose was added to the medium.

*Measurement of Radioactivity.* Radioactivity was measured in a Nuclear-Chicago gas-flow counter. Zero-time controls of approximately 45 cpm and the counter background of 20–25 cpm were subtracted from total counts in each experiment. Paper electrophoresis strips were scanned in an Actigraph gas-flow counter made by the same company. Radioactive areas on thin-layer plates and paper electrophoresis strips were located by autoradiography using Kodak No-Screen Medical X-Ray film and leaving the sheets in contact with the film for 1 week or more.

*Cell-Free Preparation.* All operations were performed at 0–4°. Several variations of the preparation procedure were tried with cells of strain 8185, and the following, a modification of that used in preparing the gramicidin S synthesizing (Bhagavan *et al.*, 1966) was found to be the best. The cells were washed twice with 0.02 M magnesium acetate, followed by one washing with 0.02 M magnesium acetate in 0.02 M mercaptoethanol (solution A). The packed cells were then suspended in two volumes of solution A and sonicated at 60 W and 20 kcycles for 10 min. Five periods of sonication each 2 min long were used, and the cells were chilled to 4° between periods. Unbroken cells and debris were removed by centrifugation at 30,000*g* for 45 min and the supernatant phase was further centrifuged at 105,000*g* for 1 hr. Unless otherwise indicated, 1 ml of 105,000*g* supernatant (hereafter referred to as sonic extract) was used per reaction tube in the tyrocidine biosynthesis experiments. Protein content was determined by the biuret method. The methods of preparation of extracts and incubation conditions used with strain 10068 were similar to those described for strain 8185. Optimal conditions for peptide synthesis by this strain will be described in a forthcoming paper (K. R. Rao and J. B. Hall, in preparation).

*Incubation Procedure.* The following conditions were found to be optimal for peptide biosynthesis in the cell-free system from *B. brevis* 8185. The incubation mixture adjusted to a final volume of 2 ml was incubated for 3 hr at 37°. The standard mixture contained the following in each tube: 1 ml of sonic extract (5–10 mg of protein), 10 μmoles of ATP, 5 μmoles of PEP, 20 μg of pyruvic kinase, 20 μmoles of magnesium acetate, 20 μmoles of mercaptoethanol, 100 μmoles of phosphate buffer (pH 7.0), and 0.5 μCi of one of the amino acids occurring in the peptides, labeled with $^{14}C$, plus 1 μmole of each of the other amino acids found in the peptides. This system was routinely used unless otherwise indicated.

*Isolation of Peptides.* The peptides were isolated by the method previously described for gramicidin S (Bhagavan *et al.*, 1966).

*Chromatography.* One-dimensional chromatography on thin-layer silica gel plates was carried out using the upper phase of 1-butanol–acetic acid–water (101:24:101, v/v) mixture as the solvent. The chromatograms were sprayed with 0.2% (w/v) ninhydrin solution in acetone to detect the peptides.

*Electrophoresis.* Electrophoresis was carried out on water-cooled plates with 50% acetic acid as the solvent. The samples were applied to the center of an 18 × 58 cm strip of Whatman No. 3MM paper and subjected to a current of 18 mA/cm at a voltage of 34 V/cm for 90 min. The dried strip was dipped in a 1% solution of bromophenol blue in water and washed in 1% acetic acid to remove excess dye. Gramcidins were identified by their electrophoretic mobility and made visible by immersing the strip in 7 N $HNO_3$ and then in water, a procedure which stains these peptides yellow (Okuda *et al.*, 1963).

*Labeling of Peptides in Whole Cells.* Twenty hours before harvest, 0.1 μCi of a uniformly labeled amino acid mixture was added/ml of culture. At harvest, the cells were precipitated by the addition of one-fifth volume of 50% trichloroacetic acid to the culture and the peptide fraction was isolated as before.

## Results

*Cultivation of B. brevis 8185.* To determine the growth phase at which the maximal rate of peptide synthesis occurred, the cells were harvested at different stages of growth and the extent of incorporation of labeled amino acid into peptide by cell-free extracts was measured. The results are reported in Table I. At optical densities of 1.19–1.33, which correspond to the late-log phase of growth of these cultures, peptide synthesis was most active. The medium must be supplemented with glucose

TABLE I: Cell Growth and Tyrocidine Biosynthesis.[a]

| Cell Density at Harvest (optical density at 650 mμ) | Radioactivity of Labeled Peptide (cpm/10 mg of protein) |
|---|---|
| 0.480 | 47 |
| 1.050 | 618 |
| 1.190 | 20,130 |
| 1.290 | 18,900 |
| 1.330 | 26,880 |
| 1.400 | 17,000 |
| 1.740 | 12,310 |

[a] The labeled amino acid was L-[U-$^{14}C$]leucine.

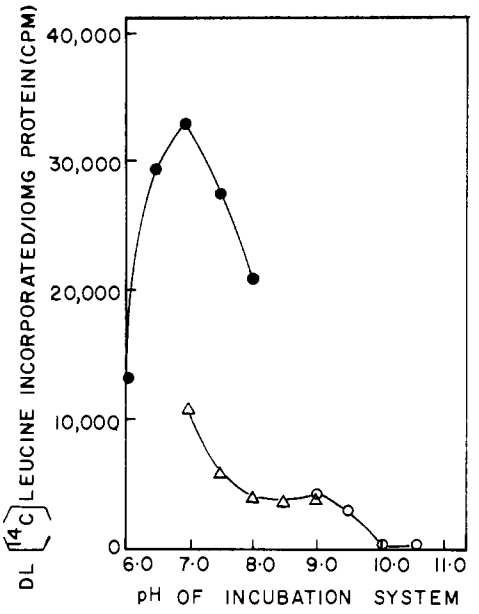


FIGURE 1: Effect of pH on the incorporation of DL-[1-$^{14}$C]-leucine into peptide. (●—●) 0.05 M phosphate buffer; (△—△) 0.01 M Tris-HCl buffer; (○—○) 0.1 M glycine–NaOH buffer.

to produce a culture which will actively produce tyrocidine. Cell-free extracts from cultures grown in the absence of glucose produced only 3% as much tyrocidine as those from glucose-grown cells as measured by the incorporation of [$^{14}$C]leucine. Okuda *et al.* (1963) observed a similar, though less striking, effect of glucose on synthesis of the antibiotic in whole cells.

*Disruption of Cells.* Aliquots of cell suspensions were sonicated for periods of 6–15 min. Cells sonicated for 10 min yielded the most active extracts, while prolonging the disruption time to 15 min reduced the activity of the extracts to 20% of the maximum value.

*Incubation Conditions.* Incorporation at the end of 0.5 hr was 22% of the maximum value and continued until a plateau was reached between 2 and 3 hr.

The relationship between pH and activity is shown in Figure 1. In phosphate buffer, the pH optimum and shape of the curve is identical with that found for the gramicidin S synthetic system (Bhagavan *et al.*, 1966). No second peak at a higher pH was observed in the Tris-HCl and glycine–NaOH buffers as had been observed for gramicidin S, although a shoulder of activity in this region is seen. No explanation for the peculiar shape of these curves has emerged from this work, although considering the multienzyme nature of the synthetic complex, such curves are not unexpected.

*Ionic Requirements.* As indicated by the pH activity curves, the synthesis of tyrocidine is greatly stimulated by phosphate. The optimum phosphate concentration was about 0.05 M at pH 7.0. A similar requirement for phosphate had been observed for gramicidin S synthesis although 0.2–0.3 M phosphate was optimal in this case (Bhagavan *et al.*, 1966). It is possible that the observed stimulation by this anion is actually due to the increase in ionic strength or a need for a polyvalent anion, since Figenschou *et al.* (1967) obtained high levels of gramicidin S synthesis by using 0.1 M Tris-HCl plus 0.1 M ammonium sulfate instead of phosphate.

Potassium did not appear to be required by this system, since varying the concentration of this ion had no effect on the activity.

Magnesium is required by the synthetic complex, but appears to be tightly bound to it. Addition of magnesium to sonic extracts or to sonic extracts that had been dialyzed against 0.02 M phosphate (pH 7.0) containing 0.02 M mercaptoethanol, for 18 hr, did not stimulate their activity. If sonic extracts were dialyzed first against 0.001 M EDTA in 0.02 M phosphate buffer (pH 7.0) containing 0.02 M mercaptoethanol, for 18 hr, and then against the same buffer lacking EDTA, approximately 80% of their synthetic activity was lost. This could be completely restored by the addition of 2 μmoles of magnesium acetate. Further additions of magnesium had no additional effect.

*Energy Sources.* In the presence of 5 μmoles of PEP and 20 μg of pyruvic kinase, maximum activity was attained with 5–10 μmoles of ATP. Activity was appreciably reduced by increasing the ATP to 20 μmoles and further reduced to 6% of the maximum by 40 μmoles of ATP. Evidently there was no appreciable ATPase activity in the extract. Table II gives further details on the energy requirements of the system. It can be seen that ATP is the essential component. Omitting PEP and pyruvic kinase had little effect on leucine incorporation, as did the addition of CTP, GTP, and UTP. It is clear, moreover, that none of the latter three nucleoside triphosphates was able to replace ATP as the energy source. Again, these findings are reminiscent of those on gramicidin S biosynthesis (Bhagavan *et al.*, 1966).

TABLE II: The Effect of Ribonucleoside Triphosphates and an Energy-Generating System for ATP on DL-[1-$^{14}$C]Leucine Incorporation into Tyrocidine.

| Energy Source | Radioactivity (cpm of peptide/10 mg of protein) |
|---|---|
| Standard system | 17,900 |
| ATP, PEP, and pyruvic kinase omitted | 115 |
| PEP and pyruvic kinase omitted | 15,370 |
| CTP, GTP, and UTP added (2 μmoles each) | 16,070 |
| ATP omitted; 10 μmoles of CTP added | 402 |
| ATP omitted; 10 μmoles of GTP added | 100 |
| ATP omitted; 10 μmoles of UTP added | 380 |

*Effect of Inhibitors.* The influence of some of the typical inhibitors of protein synthesis on the system is shown in Table III. It can be seen that none of the in-

TABLE III: The Effect of Antibiotics and Nucleases on the Incorporation of DL-[1-$^{14}$C]Leucine into Tyrocidine.[a]

| Addition (μg) | Radioactivity (cpm of peptide/10 mg of protein) | |
|---|---|---|
| | 30,000*g* Supernatant | 105,000*g* Supernatant |
| None | 12,770 | 16,840 |
| Chloramphenicol (100) | 12,660 | 13,450 |
| RNase, pancreatic (100) | 12,060 | 14,010 |
| Puromycin (100) | 12,770 | 14,880 |
| DNase I, pancreatic (100) | 11,400 | 12,820 |

[a] The incubation mixture was preincubated for 5 min before adding the energy source and [$^{14}$C]amino acid.

hibitors had any effect on peptide synthesis either in the presence or absence of ribosomes.

*Characterization of the Peptide Fraction.* The contributions of different $^{14}$C-labeled amino acids to the radioactivity of the peptide fraction are listed in Table IV. The pattern of incorporation suggests that the only peptide being formed in this system was tyrocidine D. Tyrosine is a constituent of tyrocidines A–C, but is absent from tyrocidine D in which it is replaced by tryptophan. As shown in Table IV, tyrosine did not label the peptide in this experiment. Similarly, the failure of glycine and valine to be incorporated into the peptide fraction rules out the possibility that open-chain gramicidins, of which these amino acids are constituents, were being synthesized. It is also of interest to note that the amides of glutamic and aspartic acid were incorporated directly while free aspartic acid was not incorporated at all, and the amount of glutamic acid utilized was much less than the amount of glutamine and may have represented material converted into the latter compound. Except for the relatively small amount of glutamic acid incorporated into the peptide, only amino acids found in tyrocidine D served as precursors for the peptide synthesized by these extracts.

TABLE IV: Utilization of Different $^{14}$C Compounds in the Synthesis of Peptide.[a]

| $^{14}$C-Labeled Compd | Amino Acid Sp Act. (mCi/mmoles) | Peptide (cpm/10 mg of protein) |
|---|---|---|
| DL-Leucine | 31 | 15,510 |
| DL-Tryptophan | 1.93 | 6,200 |
| L-Aspartic acid | 167 | 0 |
| L-Asparagine | 46.9 | 7,440 |
| L-Glutamic acid | 195 | 710 |
| L-Glutamine | 52.3 | 3,870 |
| DL-Phenylalanine | 4.46 | 4,580 |
| DL-Ornithine | 11 | 9,330 |
| DL-Proline | 5 | 4,870 |
| DL-Valine | 18.5 | 6,160 |
| DL-Tyrosine | 2.4 | 0 |
| L-Alanine | 117 | 0 |
| L-Serine | 120 | 0 |
| Glycine | 116 | 0 |
| Ethanolamine | 4.34 | 0 |

[a] The amino acid mixture consisted of 0.5 μCi of the specified $^{14}$C compound and 1 μmole of each of the 19 or 20 other [$^{12}$C]amino acids.

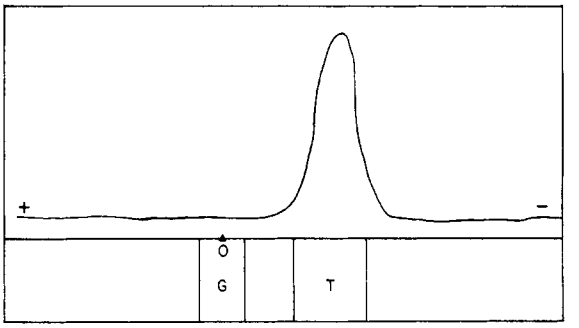


FIGURE 2: Identification of the peptides synthesized in the cell-free system. A diagram of an electrophoresis strip stained with bromophenol blue and nitric acid and scanned in a strip counter to measure radioactivity is shown. The peptides were labeled with DL-[1-$^{14}$C]leucine. "O" indicates the origin, "G" the gramicidin band, and "T" that of the tyrocidines.

The observation made above that the gramicidins did not appear to be synthesized by the cell-free system was confirmed by paper electrophoresis. Carrier gramicidins and tyrocidines were mixed with the incubation mixture and the peptide fraction was isolated and subjected to electrophoresis. After staining, the strip was scanned in the Actigraph radiation counter. Figure 2 shows the result. The gramicidins, which remain at the origin in this system, were unlabeled, while the tyrocidines, which migrate toward the cathode, contained all of the radioactivity in the peptide fraction. Fujikawa *et al.* (1966) also found that only the tyrocidines were labeled in their system. This group used a different purification procedure but one that, according to Mach *et al.* (1963) who devised it, should yield a peptide fraction containing both the tyrocidines and gramicidins.

The results of these amino acid incorporation and peptide electrophoresis experiments were rather puzzling. *B. brevis* 8185 (the one commonly referred to as the Dubos strain) has been reported to synthesize at least four tyrocidines (Battersby and Craig, 1952; Mach and Tatum, 1964) and a whole family of gramicidins (Sarges and Witkop, 1965). Our extracts appeared to synthesize only one of the tyrocidines and none of the gramicidins. It seemed possible that the cells possessed synthetic capacities that were lost during preparation of the extracts. To test this idea, the cultures were labeled for 20 hr before harvest with a mixture of radioactive amino acids, and the peptides were extracted from them. The gramicidin and tyrocidine fractions were separated by electrophoresis and scanned in the strip counter. Figure

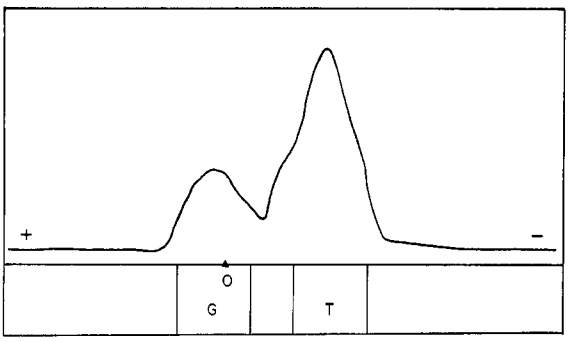


FIGURE 3: Identification of the peptides synthesized *in vivo*. A diagram of an electrophoresis strip stained with bromophenol blue and nitric acid and scanned in a strip counter to measure radioactivity is shown. The peptides were labeled with a mixture of [$^{14}$C]amino acids.

3 shows the resulting tracing, which revealed that both fractions were indeed labeled in whole cells. This experiment did not indicate whether or not more than one tyrocidine or gramicidin was formed, but did show that whole cells were able to make at least one gramicidin, while the cell-free system did not.

To confirm the failure of tyrosine to label the tyrocidine and to determine whether or not this effect was peculiar to the strain we were using, the experiments reported in Table V were carried out. It can be seen that while some tyrosine was incorporated into peptide by extracts of *B. brevis* 8185, tryptophan was incorporated much more readily. Extracts from *B. brevis* 10068, on the other hand, incorporated both amino acids quite efficiently. The two labeled aromatic amino acids had approximately the same specific activities. If it can be assumed that dilution by endogenous amino acid was similar for each of them, the distribution of label in tyrocidines formed by *B. brevis* 10068 extracts was consistent with the idea that roughly equal amounts of the four tyrocides were being formed. However, it was clear that less than 10% of the peptides produced by extracts of *B. brevis* 8185 could have consisted of tyrocidines

TABLE V: The Incorporation of Three $^{14}$C-Labeled Amino Acids into Tyrocidines by Cell-Free Extracts from Two Different Strains of *B. brevis*.[a]

| Strain | Expt | Label in Peptide (cpm/4 mg of protein) Contributed by [$^{14}$C]Tyr | [$^{14}$C]Trp | [$^{14}$C]Leu |
|---|---|---|---|---|
| ATCC | 1 | 2840 | 5100 | 4200 |
| 10068 | 2 | 2775 | 5438 | 4436 |
| ATCC | 1 | 204 | 5240 | 4700 |
| 8185 | 2 | 149 | 4500 | 4134 |

[a] The amino acid mixture consisted of 0.5 μCi of the [$^{14}$C]amino acid and 1 μmole each of the other 19 [$^{12}$C]amino acids. No gramicidins were present in the labeled peptide fraction as determined by electrophoresis.

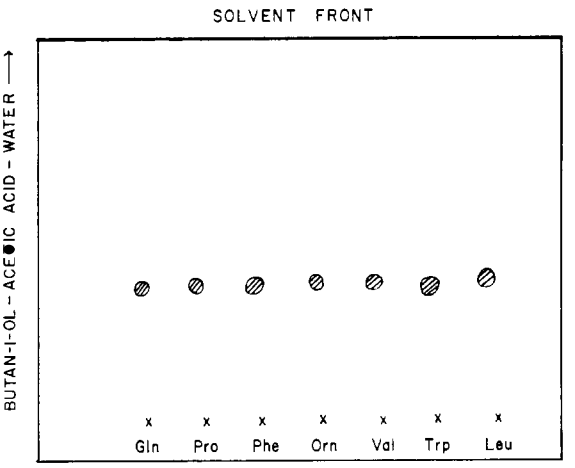


FIGURE 4: An attempt to observe peptide intermediates in tyrocidine biosynthesis. A radioautograph of a thin-layer chromatogram developed in 1-butanol–acetic acid–water is shown. "X" marks the origin for each peptide and the [$^{14}$C]-amino acid used to label that peptide is indicated below the origin.

A–C, and that at least 90% must have been tyrocidine D, if the same assumptions are made about this system.

In order to demonstrate more conclusively that the peptide formed by these extracts was largely tyrocidine D, the following experiment was performed. Peptide labeled with [$^{14}$C]tryptophan was passed through a 1 × 150 cm column of Sephadex G-25 in 10% acetic acid as described by Ruttenberg and Mach (1966). Commercial tyrocidine was added as a carrier. Three peaks showing absorbancy at 280 mμ emerged from the column, followed by a peak of radioactive material. According to Ruttenberg and Mach, tyrocidines A–D should elute from the column in that order. Apparently the commercial tyrocidine contained tyrocidines A–C so that our tyrocidine, which eluted in the relative position expected for tyrocidine D, emerged after the carrier material. The identification of these peaks was confirmed by passing [$^{14}$C]tyrosine-labeled peptide, prepared with cell-free extracts of *B. brevis* 10068, through the column with the carrier. As expected, the first three peaks were labeled, but the last peak, which should contain no tyrosine if it is indeed tyrocidine D, was unlabeled.

*Intermediate Peptides.* Besides the major product, gramicidin S, of the peptide-synthesizing system from *B. brevis* ATCC 9999, a minor component that was labeled by all of the amino acids found in gramicidin S was observed (Bhagavan *et al.*, 1966). This peptide appeared to be an intermediate in gramicidin S biosynthesis and proved to have a number of interesting properties (Pollard *et al.*, 1968). In order to determine whether or not a similar free intermediate occurred in the tyrocidine system, aliquots of the incubation mixture labeled with different amino acids were subjected to thin-layer chromatography in the 1-butanol–acetic acid–water solvent. The results, shown in Figure 4, revealed that no free intermediates with properties permitting them to be carried through our purification procedure with the tyrocidines were labeled by any of the amino acids tested.

## Discussion

The culture conditions that are optimal for tyrocidine synthesis by *B. brevis* ATCC 8185 are somewhat different from those needed for gramicidin S biosynthesis by *B. brevis* ATCC 9999. The requirements for synthesis of the cyclic decapeptides tyrocidine D and gramicidin S are very similar in cell-free extracts of the two strains of bacteria, however. Both have similar pH optima and ionic requirements, and both systems specifically require ATP and utilize it efficiently for peptide synthesis over a period of several hours without any need for an exogenous energy-generating system.

The failure of any of the specific inhibitors of protein synthesis to reduce peptide synthesis, and the lack of a requirement for ribosomes or tRNA, appears to rule out the possibility that a protein-synthesizing system is responsible for the production of tyrocidine. The absence of gramicidin biosynthetic activity from these extracts might permit the reservation that these peptides could be formed by a ribosomal system. This possibility is being investigated.

The observation that only tyrocidine D appeared to be synthesized in our extracts was unexpected. Mach and Tatum (1964) had observed that the synthesis of tyrocidines by whole cells was readily influenced by the amino acid composition of the medium, with phenylalanine promoting the synthesis of tyrocidine A, which contains three residues of this amino acid and a single tyrosine but no tryptophan, while addition of tryptophan to the culture led to the formation primarily of tyrocidine D, containing three tryptophans, no tyrosine, and but one phenylalanine. They suggested that these results could best be explained by assuming that the enzymatic system making these peptides was partially nonspecific and would substitute tryptophan for phenylalanine or tyrosine in certain positions of the peptide chain. The results reported here seemed to cast some doubt on this hypothesis. Under the standard incubation conditions that we employed, tyrocidine D was the principal peptide synthesized, and little tyrosine, which is found in tyrocidines A–C, was incorporated, even though phenylalanine and tyrosine were present in the incubation mixture in amounts equal to those of tryptophan. K. Kurahashi (personal communication) has informed us, however, that the studies of this system by his group suggest that the enzyme system will incorporate phenylalanine and tyrosine if they are added in sufficient concentration, but that tryptophan utilization is favored. If this finding is confirmed, it would tend to support the hypothesis set forth by Mach and Tatum on the mechanism of biosynthesis of the different cyclic antibiotic polypeptides produced by these bacteria.

Our own comparative studies on peptide synthesis in the two strains of bacteria indicate that all four tyrocidines are probably formed by both, but that *B. brevis* 8185 has a decided bias in favor of tyrocidine D biosynthesis, at least under the conditions here employed, while the *B. brevis* 10068 strain used by Mach and Tatum shows no such tendency. The latter appears to respond more readily, therefore, to alterations in the availability of amino acids.

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