NON-RIBOSOMAL BIOSYNTHESIS OF LINEAR GRAMICIDINS

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Received 11 November 1971

1. Introduction

In addition to tyrocidine which is a cyclic decapeptide, linear peptides containing 15 amino acids are produced by the same strain of *Bacillus brevis*. These are the gramicidin A, B and C with the following structures [1]:

(L-Ileu)
N-formyl → L-Val → Gly → L-Ala → D-Leu → L-Ala → D-Val → L-Val → D-Val
↓ A
Ethanolamine ← L-Try ← D-Leu ← L-Try ← D-Leu ← L-Try ← D-Leu ← L-Try
(L-Phe) B
(L-Tyr) C

It is now well established that the biosynthesis of the cyclic peptides gramicidin S and tyrocidine [2] are synthesized independently of ribosomes and it has been suggested that the linear gramicidins are synthesized by a similar mechanism [3]. However, since no cell free system for the study of the synthesis of this peptide is yet available, it was considered of interest to investigate in whole cells if the synthesis was independent of ribosomes.

In the present work the synthesis of the linear gramicidins was studied in whole cells in the presence of chloramphenicol. The results clearly indicate that the synthesis is independent of ribosomes.

2. Methods and materials

2.1. *Cultivation of bacteria*

B. brevis ATCC 8185 was maintained on milk agar slants and grown under aeration in a milk–yeast extract medium [4] in connicals in a Gyrotory incubator shaker at 37°. The density of the culture was measured at 650 nm in 10 mm cells in a Spectronic 20 spectrophotometer.

2.2. *Isolation of ^{14}C-labelled linear gramicidins and tyrocidines*

200 mg of ^{14}C-L-valine were added to 10 ml of culture and the pH adjusted to 4.0 by the addition of N-HCl. The precipitate was washed once with 2% NaCl (5 ml, pH 4.0). The residue was extracted with ethanol (5 ml) for 24 hr at room temp and finally washed twice with ethanol (1 ml each time). The extract was evaporated to dryness and dissolved in 1 ml of ethanol. To purify the peptides, the extract was put on an acid alumina column (Woelm Acid, grade IV, 0.6 × 2 cm) and washed with ethanol. The eluate contained a mixture of tyrocidines and linear gramicidins. To separate linear gramicidins from tyrocidines, the mixture of the peptides was washed through a Dowex 50 × 4 (hydrogen form) in ethanol. The tyrocidines which are basic were retained by the column while the linear gramicidins which are neutral passed

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through. Thin layer chromatography on silica plates (medium H according to Stahl, E. Merck AG, Darmstadt) in ethylacetate–pyridine–acetic acid –water (60:20:6:11, by vol) of the peptide fractions revealed after radiography only spots corresponding to tyrocidine (R_F 0.40) and linear gramicidins (R_F 0.95).

2.3. *Determination of protein*

Protein was determined by a method described earlier [5].

2.4. *Radiochemicals and measurement of radioactivity*

Uniformly labelled ^{14}C-L-valine (200 μCi/μmole) was purchased from New England Nuclear Corp., Mass., USA. Radioactive samples were counted in a Frieseke and Hoepfner windowless flow counter.

2.5. *Authentic peptides*

Tyrocidine was obtained from Nutritional Biochemical Corporation, Cleveland, Ohio, and gramicidin A was a gift from Dr. Erhard Gross, Department of Health, Education and Welfare, National Institute of Health, Bethesda, Maryland, USA.

3. Results and discussion

Previous work had shown that in the *B. brevis* strain ATCC 9999 chloramphenicol at 100 μg/ml effectively inhibits protein synthesis without affecting gramicidin S formation [5]. In the present experiment the same concentration was used. Chloramphenicol was added to the growing culture when tyrocidine and linear gramicidin synthesis were maximal. It is seen from table 1 that when ^{14}C-L-valine was added to the culture 15 min after the addition of chloramphenicol, only negligible incorporation took place into protein as compared to the control culture. However, in the case of the linear gramicidins, incorporation took place in the presence of chloramphenicol as was the case for tyrocidine. In both cases the incorporation into the peptides was higher in the chloramphenicol treated cultures, the reason being that more labelled valine is available for peptide synthesis when protein synthesis is inhibited.

Table 1

The effect of chloramphenicol on the synthesis of linear gramicidin. Radioactivity (counts/min/ml culture) 1 hr after addition of ^{14}C-valine.

	In protein	In linear gramicidins	In tyrocidines
Control culture	40,000	18,000	24,000
Presence of chloramphenicol	1,000	20,000	32,000

A 1-liter flask containing 250 ml medium was inoculated with *B brevis*. The flask was shaken at 37° until the pH in the medium reached 7.2 (optical density 2.0). 100 ml culture was transferred to each of two 1-liter flasks and chloramphenicol (100 μg/ml) was added to one of the flasks. 15 min later 12.5 μCi ^{14}C-L-valine was added to both cultures and radioactivity in protein, linear gramicidins and tyrocidines was determined.

The present experiments demonstrate that the 15 amino acid long linear gramicidins are produced independently of ribosomal protein synthesis.

References

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