Comparison of the Effect of Linear Gramicidin Analogues on Bacterial Sporulation, Membrane Permeability, and Ribonucleic Acid Polymerase†

Henry Paulus,* Nilima Sarkar, Pranab K. Mukherjee, Donna Langley, V. T. Ivanov, E. N. Shepel, and W. Veatch*

ABSTRACT: Various analogues of linear gramicidin were tested for their biological activity in restoring the normal spore phenotype of gramicidin-negative mutants of *Bacillus brevis* and for their ability to increase cation conductivity of black lipid membranes and to inhibit bacterial RNA polymerase. Whereas many biologically active gramicidin analogues had no effect on membrane permeability, all biologically active peptides were able to inhibit ribonucleic acid (RNA) polymerase. These observations make it unlikely that membranes are the site of action of gramicidin during bacterial sporulation, but they are consistent with the notion that gramicidin functions to control RNA synthesis during the transition from vegetative growth to sporulation (Sarkar & Paulus, 1972). The relationship between peptide structure and the ability to restore normal sporulation and inhibit RNA polymerase showed that the eight amino-terminal residues have little influence on the function of gramicidin, whereas the highly nonpolar repeating sequence D-leucyl-L-tryptophan is essential for biological activity and may represent the site of interaction with RNA polymerase.

Our earlier finding that gramicidin-negative mutants of *Bacillus brevis* are unable to form normal spores unless provided with gramicidin has clearly established a biological function of the peptide antibiotic in the sporulation process (Mukherjee & Paulus, 1977). However, this raises an important question as to the mechanism by which gramicidin exerts its biological effect. Gramicidin can act as an antibacterial agent by affecting the cation permeability of membranes (Harold & Baarda, 1967), a property which has been studied extensively [e.g., Chappell & Crofts (1965), Harris & Pressman (1967), Hladky & Haydon (1972), Veatch et al. (1975) and Veatch & Stryer (1977)]. On the other hand, we have found that the antibiotic can specifically inhibit transcription with purified bacterial RNA polymerase (Paulus & Sarkar, 1976; Sarkar et al., 1977). In this paper, we report studies with a series of gramicidin analogues in which the specificity of the in vivo response of the gramicidin-negative mutant was compared with the ability to affect membrane permeability and to inhibit purified RNA polymerase. Our results eliminate the ionophoretic effect of gramicidin as the basis of its biological action during bacterial sporulation but are consistent with a biological function that involves the modulation of transcription.

Materials and Methods

Peptides. Gramicidin was obtained from Nutritional Biochemicals and consisted of approximately 85% gramicidin A, 10% gramicidin B, and 5% gramicidin C (Gross & Witkop, 1965). The gramicidin-related peptides used in this work consisted of alternating L and D amino acid residues as in gramicidin and are listed in Table I. Peptides 1–6 were prepared by modifying the end groups of gramicidin A as described by Morrow et al. (1979). Peptides 7–22 were products of chemical synthesis by the procedures of Shepel et al. (1976).

RNA Polymerase. RNA polymerase was purified from exponentially growing cells of *B. brevis* ATCC 8185 (Paulus & Sarkar, 1976) and assayed with bacteriophage ϕ_e DNA as template as described previously (Sarkar et al., 1977).

Effect of Peptides on Sporulation and Dipicolinate Synthesis. B. brevis strain M1, a gramicidin-negative derivative of *B. brevis* ATCC 8185, was isolated and cultured as described earlier (Mukherjee & Paulus, 1977). At the onset of sporulation (culture density of 250 Klett units with a no. 54 filter), gramicidin or related peptides were added as a concentrated solution in dimethyl sulfoxide and sporulation was allowed to proceed for at least 48 h. An equivalent amount of dimethyl sulfoxide (final concentration of 1%) was also added to control cultures. Samples of the sporulated culture were heated at 80 °C for 20 min or 3 h and then plated on Penassay agar (Difco) to determine the frequency of viable spores as colony-forming units. Another sample of the culture was assayed for dipicolinic acid content by the method of Janssen et al. (1958).

Assay of Channel-Forming Ability. The ability of gramicidin and related peptides to form ion channels in black lipid bilayer membranes was measured as described by Veatch & Stryer (1977) and Morrow et al. (1979).

Results

A gramicidin-dependent property of *B. brevis* that lends itself most readily to quantitation is the production of dipicolinic acid. The spores of gramicidin-negative mutants have ~20% of the normal dipicolinate content, but the addition of gramicidin at the onset of sporulation leads to a threefold increase (Mukherjee & Paulus, 1977). Measurement of dipicolinate production thus permits the quantitative assessment of the biological activity of gramicidin and related

† From the Department of Metabolic Regulation, Boston Biomedical Research Institute, Boston, Massachusetts 02114 (H.P., N.S., P.K.M., and D.L.), the Departments of Biological Chemistry (H.P. and N.S.) and Pharmacology (W.V.), Harvard Medical School, Boston, Massachusetts 02115, and the Shemyakin Institute of Bioorganic Chemistry, Moscow 117312, USSR (V.T.I. and E.N.S.). *Received April 27, 1979.* This is paper 3 in a series entitled "Biological Function of Gramicidin". Papers 1 and 2 are Mukherjee & Paulus (1977) and Sarkar et al. (1977), respectively. This work was supported by Research Grants GM 23149 from the National Institute of General Medical Sciences (H.P.) and AI 15311 from the National Institute of Allergy and Infectious Diseases (W.V.) and by grants from the Pharmaceutical Manufacturers Association (W.V.), The Medical Foundation (W.V.), and the Milton Fund of Harvard University (W.V.). W.V. is the recipient of Research Career Development Award AI 00320 from the National Institute of Allergy and Infectious Diseases, and P.K.M. was supported by a Research Fellowship from The Medical Foundation, Inc., Boston, MA.

0006-2960/79/0418-4532$01.00/0

Table I: Methods of Preparation of Peptides Used

no.	peptide	ref
1	HCO-L-Cys-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH	Morrow et al. (1979)
2	HCO-L-Cys(Me)-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH	Morrow et al. (1979)
3	Ac-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH	Sarges & Witkop (1965)
4	L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH	Ishii & Witkop (1964)
5	Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH	Morrow et al. (1979)
6	HCO-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH	Morrow et al. (1979)
7	HCO-L-Val-Gly-L-Val-D-Val-L-Trp-(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
8	Z-L-Val-(D-Leu-L-Trp)$_4$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
9	HCO-L-Val-Gly-L-Trp-(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
10	HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-(D-Leu-L-Trp)$_2$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
11	HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-(D-Leu-L-Trp)$_2$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
12	HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Leu-L-Trp-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
13	HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
14	Z-(D-Leu-L-Trp)$_2$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
15	Z-D-Leu-L-Trp-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
16	Z-(D-Leu-L-Trp)$_4$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
17	Z-L-Trp-(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
18	Z-L-Val-D-Val-L-Trp-(D-Leu-L-Trp)$_2$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
19	Z-(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
20	Z-L-Trp-(D-Leu-L-Trp)$_2$-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
21	Z-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-NH(CH$_2$)$_2$OH	Shepel et al. (1976)
22	Z-L-Val-D-Val-L-Trp-D-Leu-L-Trp-NH(CH$_2$)$_2$OH	Shepel et al. (1976)

peptides. We took advantage of this fact to study the relationship of structure to biological function of various peptides related to gramicidin. For convenience, the peptides used in these studies were divided into the following groups on the basis of their structural difference from gramicidin: group I, replacement of the amino-terminal residue or the amino-terminal protecting group; group II, absence of an amino-terminal protecting group; group III, deletion of one or more residues in positions 1–6 but not in other positions; group IV, deletion of one or more residues in positions 7–10 but not in higher positions; and group V, deletion of one or more residues in positions 11–15. The results of experiments with 15 gramicidin analogues are summarized in Table II. The peptides of groups I and III were as effective as gramicidin in restoring the ability to produce dipicolinate, the peptides of group V exhibited no significant activity, those of group II had either a much reduced effect (peptide 4) or no effect (peptide 5), and the response to peptides of group IV was variable, peptide 9 being inactive and peptide 10 being at least as effective as gramicidin. To verify that the observed effects on dipicolinate production were indeed related to altered heat resistance of the bacterial spores, the effect of some gramicidin analogues on spore heat stability was also studied. As shown in Table III, increased heat stability in response to peptide addition correlated well with stimulation of dipicolinate production. It should also be noted that two of the peptides (peptides 5 and 9) severely inhibited sporulation. Their failure to stimulate dipicolinate formation could thus be due to a general inhibitory effect.

When the ability of the 15 peptides to affect ion conductance of black lipid membranes was examined, only one of them (peptide 2) was seen to have a major effect, two (peptides 3 and 6) had a slight activity, and all others were inactive as ionophores (Table II). Thus, there was clearly no connection between membrane channel forming capacity and the biological activity of the gramicidin analogues. A much better correlation was observed between the ability of gramicidin analogues to stimulate dipicolinate production by the gramicidin-negative mutant and their ability to inhibit purified bacterial RNA polymerase. The peptides of groups I–III were as effective if not better inhibitors of RNA polymerase than gramicidin itself, peptides of group IV were slightly less inhibitory than gramicidin, and those of group V were completely inactive (Table II). With the exception of peptides 5 and 9,

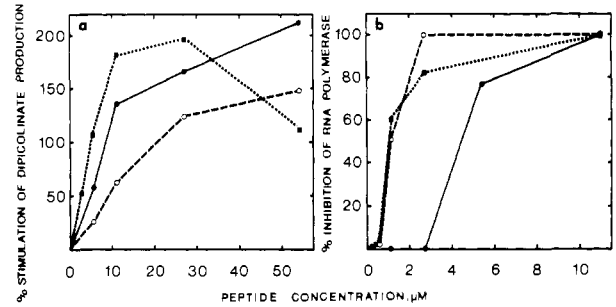

FIGURE 1: Effect of gramicidin and related peptides on (a) dipicolinate production and on (b) RNA polymerase. The effect on dipicolinate production by the gramicidin-negative mutant *B. brevis* M1 was studied as outlined under Materials and Methods. Dipicolinate produced in the absence of added peptide was 80 nmol/mL of culture (SD = 3 nmol/mL). Inhibition of purified RNA polymerase from *B. brevis* was assayed as described by Sarkar et al. (1977) with bacteriophage ϕ_e DNA (0.001 A_{260}/mL) as template. The peptides were dissolved in dimethyl sulfoxide, and the final concentration of dimethyl sulfoxide in all incubations (including controls) was 5%: (●) gramicidin; (○) peptide 4; (■) peptide 8.

which had no biological effect in vivo but could inhibit RNA polymerase effectively, there was at least qualitative agreement between these two activities. It should be noted that there was no instance of a peptide which showed a biological effect but was not an inhibitor of RNA polymerase in vitro.

The effects of peptide concentration on the synthesis of dipicolinate and on RNA polymerase inhibition are compared in Figure 1. Whereas the relative effectiveness of gramicidin and the synthetic analogue peptide 8 was similar for both processes, desformyl-gramicidin (peptide 4) was relatively less active when tested in the whole cell system (Figure 1a) than when tested with purified RNA polymerase (Figure 1b). The much lower concentration at which all peptides produced inhibition of RNA polymerase is due to the conditions chosen for the assay of RNA synthesis. As described in the accompanying paper (Sarkar et al., 1979), inhibition of RNA polymerase by gramicidin is competitive with DNA; accordingly, the concentration required for 50% inhibition is a function of DNA concentration. In the experiments described here, very low concentrations of DNA were used to increase the apparent sensitivity to inhibition by gramicidin and thereby avoid artifacts that might arise at high peptide concentrations due to their limited solubility.

Table II: Effect of Gramicidin and Related Peptides on Dipicolinate Production, RNA Polymerase, and Bilayer Membranes

group	compd	peptide (1 2 3 4 5 6 7 8 9 10 11 12 13 14 15)	% stimulation of DPA production in *B. brevis* M1 by 11 μM peptide[a]	% inhibn of *B. brevis* RNA polymerase at peptide concn of 2.7 μM	11 μM	27 μM[b]	channel-forming capacity in black bilayer membrane[c]
	gramicidin	F-Val-Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	130	0	100	100	1.0
I	peptide 1	F-*Cys*-Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	146	76	80	72	<0.001
I	peptide 2	F-*Cys*(*Me*)-Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	122		74	91	0.1
I	peptide 3	*Ac*-Val-Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	145	22	99	100	0.03
II	peptide 4	Val-Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	62	100	100	100	<0.001
II	peptide 5	Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	20	60	100	92	<0.001
III	peptide 6	F- - - -Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	120	45	99	99	0.01
III	peptide 7	F-Val-Gly- - - - - - - - - - - - - -Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	192	0	76	88	<0.001
III	peptide 8	Z- - - - - - - - - - - - - - - - - - - -Val-*Leu*-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	181	88	98	97	<0.001
IV	peptide 9	F-Val-Gly- - - - - - - - - - - - - - - - - - - -Trp-Leu-Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	25	0	43	87	<0.001
IV	peptide 10	F-Val-Gly-Ala-Leu-Ala-Val-Val-Val- - - - - - - -Trp-Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	173	0	11	85	<0.001
V	peptide 11	F-Val-Gly-Ala-Leu-Ala- - - - - - - - - - - - - - - - - - - -Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	23	10	−29	−27	<0.001
V	peptide 12	F-Val-Gly-Ala-Leu-Ala- -Leu-Trp-NHCH$_2$CH$_2$OH	20	0	0	0	<0.001
V	peptide 13	F-Val-Gly-Ala-Leu-Ala-Val-Val-Val- - - - - - - - - - - - - - - - - - - -Trp-NHCH$_2$CH$_2$OH	28	0	0	0	<0.001
V	peptide 14	Z- -Leu-Trp-Leu-Trp-NHCH$_2$CH$_2$OH	38	0	0	0	<0.001
V	peptide 15	Z- -Leu-Trp-NHCH$_2$CH$_2$OH	30	0	0	0	<0.001

[a] Dipicolinate (DPA) production in the absence of added peptide was 80 nmol/mL (SD = 20 nmol/mL or 25%). [b] Purified enzyme assayed with bacteriophage ϕ_e DNA (0.001 A_{260}/mL) as template. [c] Conductance relative to gramicidin.

Table III: Effect of Gramicidin and Related Peptides on the Heat Stability of Spores of *B. brevis* Strain M1

peptide added[a]	viable spores after 20 min at 80 °C (spores/mL) (×10^6)	viable spores after 3 h at 80 °C (spores/mL) (×10^6)	ratio of survivors at 3 h and 20 min
none	1020	90	0.09
none	1080	88	0.08
11 μM gramicidin	1140	580	0.51
54 μM gramicidin	1000	387	0.39
54 μM peptide 4	1000	352	0.35
11 μM peptide 5	18	2	0.11
11 μM peptide 8	820	345	0.42
11 μM peptide 9	11	1	0.10
11 μM peptide 14	700	89	0.13
11 μM peptide 15	720	85	0.12

[a] The peptides were added as solutions in dimethyl sulfoxide at a culture density of 250 Klett units. The final concentration of dimethyl sulfoxide in the cultures (including the control) was 1%.

In order to study in greater detail the relationship between peptide structure and biological function, we examined the ability to stimulate dipicolinate production and to inhibit RNA polymerase of a series of synthetic peptides related to the carboxyl-terminal sequence of gramicidin. As shown in Figure 2, the biological activity increased progressively with the number of amino acid residues and attained a level comparable to that of gramicidin at the heptapeptide stage.

Discussion

A common problem encountered in the study of the biological function of a natural product is the correlation of its in vitro effects with its mode of action in the intact organism. This problem is especially acute in the case of the peptide antibiotic gramicidin, which can exert two quite different biochemical effects: the formation of membrane channels, leading to increased permeability to monovalent cations, and the inhibition of bacterial RNA polymerase, leading to an altered specificity of transcription. However, the fact that the physiological response to gramicidin could be measured in terms of its ability to restore a normal spore phenotype in gramicidin-negative mutants gave us the opportunity to compare the in vivo action of various gramicidin analogues with their in vitro activities. Such a comparison should permit a decision as to which of the in vitro modes of action, if any, is responsible for biological activity.

Several factors must be kept in mind when interpreting the results of experiments of this sort. The first is the fact that the action on whole cells is necessarily more complex than that in a defined in vitro system, as it may involve additional steps such as the uptake of the antibiotic by the cells. As a result, a particular peptide may show an effect in a cell-free system but be inactive when tested with whole cells because it cannot reach its physiological target. For this reason, the inability of a peptide, active in an in vitro assay system, to exert a biological effect cannot be taken as a decisive result. On the other hand, one would expect that any peptide which can affect sporulation should also show an effect in the in vitro assay system; if it does not, the in vitro activity studied must be unrelated to the physiological target. A second reservation concerns the quantitative interpretation of the results. The solubility properties of the gramicidin analogues may have different consequences when the interaction with whole cells or the effect on a soluble enzyme system is studied. For example, an ionic derivative might not be readily taken up by the cells but might have a greater inhibitory effect on purified RNA polymerase on account of its greater solubility in water.

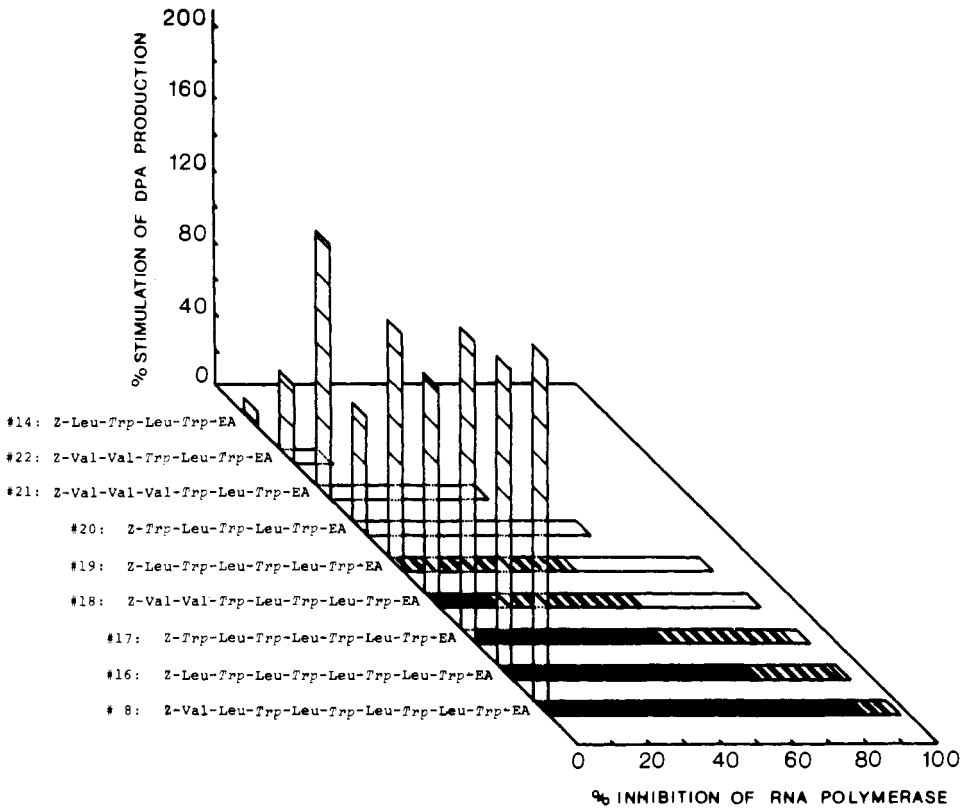

FIGURE 2: Effect of short synthetic peptides on dipicolinate production and on RNA polymerase. The conditions of the experiment were as described in the caption to Figure 1. The effect on dipicolinate production by *B. brevis* strain M1 (vertical axis) was studied at a peptide concentration of 11 μM, and inhibition of *B. brevis* RNA polymerase (horizontal axis) was studied at concentrations of 2.7 (■), 11 (▨), and 27 μM (□). Abbreviations used: EA = $NH(CH_2)_2OH$; DPA = dipicolinate.

Table IV: Summary of Biochemical Effects of Gramicidin Analogues

peptide group	ability to restore normal sporulation	ability to inhibit RNA polymerase	ability to form membrane channels
I	high activity	high activity	variable activity
II	low or no activity	high activity	no activity
III	high activity	high activity	no activity
IV	variable activity	low activity	no activity
V	no activity	no activity	no activity

Hence, an exact quantitative correspondence between in vitro and in vivo activity may not necessarily be expected. A third complication can arise from unspecific side effects of the peptide on intact cells. The inhibition of any process required for growth would obviously mask a specific stimulatory effect on sporulation.

The results of our experiments are summarized qualitatively in Table IV. Many gramicidin analogues which were as effective as gramicidin in restoring the normal spore phenotype (groups I and III) were unable to form membrane channels. This observation clearly eliminates the ionophoretic properties of gramicidin as the basis for its biological activity. On the other hand, the agreement between biological activity and inhibition of RNA polymerase was much better. All peptides that could restore the normal spore phenotype were also inhibitors of RNA polymerase. In fact, with the peptides of groups I, III, and V there was a good quantitative correlation between the two activities. The peptides of group II were very effective inhibitors of RNA polymerase but had smaller effects on bacterial sporulation. This quantitative discrepancy may be due to the fact that these peptides, which lack an amino-terminal blocking group, are cationic and therefore may not be taken up as readily by the intact cell. Another apparent discrepancy was seen in group IV, where peptide 9 had no significant effect upon dipicolinate production and yet was an inhibitor of RNA polymerase. However, as shown in Table II, peptide 9 (and also peptide 5) strongly inhibited overall sporulation, probably due to a secondary growth-inhibitory effect, which may well have obscured a specific stimulatory effect on dipicolinate synthesis. In general, therefore, our results are consistent with the idea that the effect of gramicidin on sporulation is mediated through its interaction with RNA polymerase. On the other hand, the well-known antibacterial activity of gramicidin, mediated through its action on bacterial membranes, appears to be irrelevant to the biological function of the peptide.

The studies with the gramicidin analogues also provided information concerning the structural features of the gramicidin molecule that are essential for its function. The amino-terminal protecting group and residues 1–6 had relatively little influence on the ability of gramicidin to exert its effect on sporulation and to inhibit RNA polymerase, and even deletion of residues between positions 7–10 did not completely abolish its activity. In contrast, the integrity of the five carboxyl-terminal residues of gramicidin was essential for its function. The tetrapeptide Z-(D-Leu-L-Trp)$_2$-$NH(CH_2)_2OH$ was without significant biological activity, while the pentapeptide Z-L-Trp-(D-Leu-L-Trp)$_2$-$NH(CH_2)_2OH$ was able to stimulate dipicolinate formation and to inhibit RNA polymerase and the heptapeptide Z-L-Trp-(D-Leu-L-Trp)$_3$-$NH(CH_2)_2OH$ had a biological activity comparable to that of gramicidin. In every case, lengthening the peptide chain with nonpolar amino acid residues enhanced biological activity, the alternating sequence L-tryptophanyl-D-leucine being more effective than L-valyl-D-valine. The interaction with RNA polymerase may thus involve the highly hydrophobic repeating sequence D-leucyl-L-tryptophan. This is of interest in light of

the finding, described in the accompanying paper (Sarkar et al., 1979), that gramicidin interacts with the DNA binding site of RNA polymerase. Finally, it should be noted that the heptapeptide Z-L-Trp-(D-Leu-L-Trp)$_3$-NH(CH$_2$)$_2$OH, the octapeptide Z-(D-Leu-L-Trp)$_4$-NH(CH$_2$)$_2$OH, and the nonapeptide Z-L-Val-(D-Leu-L-Trp)$_4$-NH(CH$_2$)$_2$OH are more effective than gramicidin in restoring the normal spore phenotype and in inhibiting RNA polymerase. The fact that completely synthetic oligopeptides can effectively substitute for the natural product is of considerable interest and opens the way for the development of affinity-label probes to study the mechanism of action of gramicidin both in vivo and in vitro.

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Studies on the Mechanism and Specificity of Inhibition of Ribonucleic Acid Polymerase by Linear Gramicidin†

Nilima Sarkar, Donna Langley, and Henry Paulus*

ABSTRACT: In order to define the mechanism by which linear gramicidin modulates transcription during sporulation of *Bacillus brevis*, we have examined the kinetics and the specificity of inhibition of ribonucleic acid (RNA) synthesis by the peptide antibiotic. The inhibition by gramicidin was competitive with respect to the duplex deoxyribonucleic acid (DNA) template, indicating interference with the binding of DNA to the enzyme. Gramicidin inhibition was observed with both procaryotic and eucaryotic RNA polymerases, but not with DNA polymerase, suggesting that the gramicidin binding site was conserved during evolution and probably corresponds to the DNA binding site of RNA polymerase. σ factor had no significant effect on the inhibition by gramicidin. At high DNA concentrations, inhibition was incomplete even at high gramicidin concentrations. Direct evidence for a gramicidin-resistant mode of transcription was obtained by titrating the DNA template with actinomycin D. When ~75% of transcription was blocked by the drug, the residual RNA synthesis was completely refractory to inhibition by gramicidin. This effect was seen only with natural DNA templates and not with synthetic polynucleotides or denatured DNA, suggesting that it involves specific classes of DNA sequences. Unlike actinomycin D, other drugs which inhibit RNA synthesis by complexing with DNA could not modulate gramicidin inhibition. The most simple interpretation is that gramicidin inhibition occurs selectively at certain classes of promoters which are also most sensitive to blockage by actinomycin D. It appears that by interacting with the DNA binding site, gramicidin can selectively modulate the affinity of RNA polymerase for different promoters.

The studies described in the preceding paper demonstrated that the biological function of gramicidin is not due to its ionophoretic properties but seems rather to be related to its ability to inhibit RNA synthesis. It was therefore of interest to study in some detail the effect of the antibiotic on the transcription process. We had shown earlier that gramicidin inhibits the interaction of RNA polymerase with DNA but has no effect on RNA chain initiation and elongation (Paulus & Sarkar, 1976; Sarker et al., 1977). In this paper, we present a more detailed investigation on the kinetics and specificity of inhibition of RNA synthesis by gramicidin. Our results suggest that the peptide antibiotic interacts with a DNA binding site on RNA polymerase.

Materials and Methods

Enzymes. RNA polymerase was purified from exponentially growing cells of *Bacillus brevis* ATCC 8185 as described

† From the Department of Metabolic Regulation, Boston Biomedical Research Institute, Boston, Massachusetts 02114, and the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115. *Received April 27, 1969.* This is paper 4 in a series entitled "Biological Function of Gramicidin". The preceding paper in the series is Paulus et al. (1979). This work was supported by Research Grant GM 23149 from the National Institute of General Medical Sciences.

0006-2960/79/0418-4536$01.00/0