EFFECT OF GRAMICIDIN A AND VALINOMYCIN ON PRODIGIOSIN PRODUCTION BY NON-PROLIFERATING *SERRATIA MARCESCENS*

MIQUEL VIÑAS, AIDA EL-EBIARY, JESUS GUINEA and JOSE G. LOREN

Departament de Microbiologia,
Facultat de Farmacia,
Universitat de Barcelona,
08028 Barcelona, Spain

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Prodigiosin is a secondary metabolite produced by some strains of *Serratia marcescens* and other taxonomically unrelated bacteria. Some authors have reported that prodigiosin exhibits antimicrobial activity against Gram-positive bacteria, fungi and protozoa[1,2].

Non-proliferating cultures (NPC) of *S. marcescens* are unable to produce prodigiosin unless they are induced by certain amino acids such as L-proline[3]. When NPC were induced with 10 mg of L-proline/ml and simultaneously treated with polymyxin B, prodigiosin biosynthesis was enhanced[4]. By contrast, polymyxin B inhibits chromogenesis in proliferating cultures[5]. This paper describes our studies on the effect on chromogenesis in *Serratia* of two ionophores, linear gramicidin A and valinomycin, when used alone or in the case of gramicidin A simultaneously with polymyxin B.

The bacterial strain used in this study was *S. marcescens* ATCC 274. The strain was maintained on Trypticase Soy agar (TSA) (BBL) and subcultured for the experiments in Trypticase Soy broth (TSB) (BBL).

Chemicals were obtained from Fluka and were all of analytical grade. NPC were prepared according to QADRI and WILLIAMS[3]. To prevent prodigiosin production, cells were incubated at 38°C; under these conditions no pigment was formed[6]. After centrifugation twice, washed cells were incubated at 28°C in a reciprocal shaking water bath, and amino acids and/or antibiotics were added.

Protein was measured by the method of LOWRY *et al.*[7] using bovine serum albumin as standard. Prodigiosin was extracted with ethanol - HCl[8] and the absorbance measured at 535 nm in an Uvikon 810 spectrophotometer (Kontron). The amounts of prodigiosin synthesized were calculated from its specific absorbance (51.5×10^{-3} liters/g·cm).

Fig. 1. Kinetics of prodigiosin production in NPC induced with 10 mg of L-proline/ml and in the presence of different concentrations of gramicidin A.

□ 40 μg/ml, △ 20 μg/ml, ○ 10 μg/ml, ■ 5 μg/ml, ▲ 80 μg/ml, ● is a control of 10 mg of L-proline/ml, ▽ 40 μg of gramicidin A/ml without L-proline.

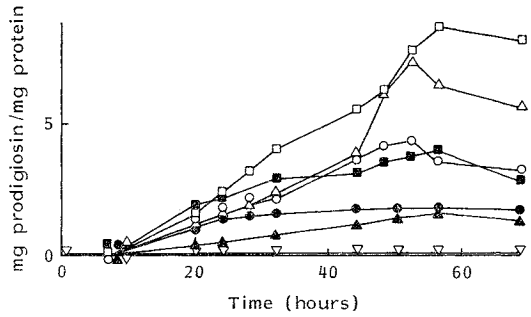


Fig. 2. Kinetics of prodigiosin production in NPC induced with 10 mg of L-proline/ml and treated with 300 μg of polymyxin B/ml together with different concentrations of gramicidin A.

○ 10 μg/ml, △ 5 μg/ml, □ 20 μg/ml, ● 40 μg/ml, ■ 40 μg of gramicidin A/ml with only L-proline, ▲ 40 μg of gramicidin A/ml alone and ▽ is 40 μg of gramicidin A/ml and 300 μg of polymyxin B/ml without L-proline.

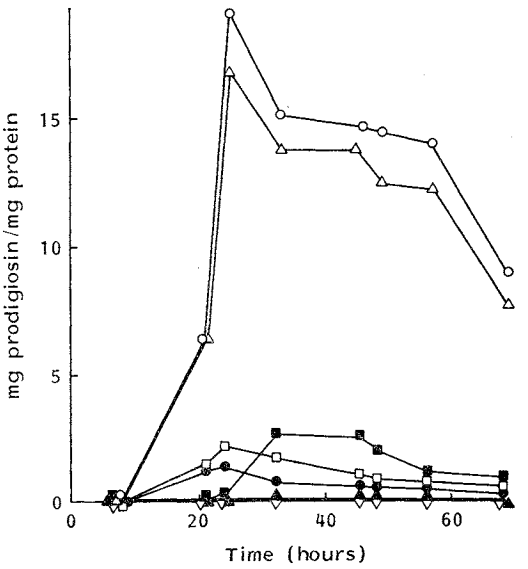
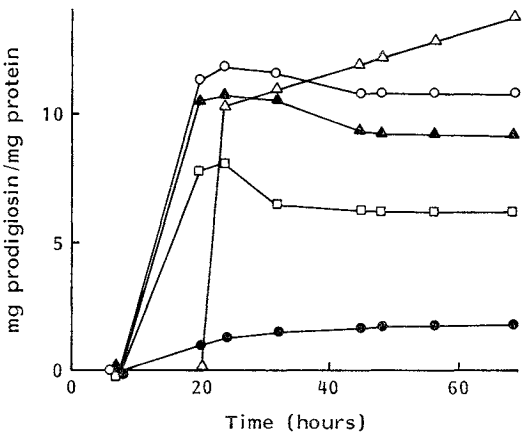


Fig. 3. Kinetics of prodigiosin production in NPC induced with 10 mg of L-proline/ml and treated with different concentrations of valinomycin.

○ 10 μg/ml, ▲ 100 μg/ml, □ 1 μg/ml, ● is a control of L-proline and △ is a control of 10 mg of L-proline/ml and 300 μg of polymyxin B/ml.



When NPC of *S. marcescens*, induced by 10 mg of L-proline/ml were treated with different concentrations of gramicidin A (below 40 μg/ml), an increase in chromogenesis was observed. Fig. 1 shows the kinetics of prodigiosin biosynthesis in NPC induced by 10 mg of L-proline/ml and in the presence of different concentrations of gramicidin A. However the biosynthesis of prodigiosin was inhibited by concentrations of gramicidin A higher than 40 μg/ml (Fig. 1). When both gramicidin A and 300 μg of polymyxin B/ml were present this inhibitory effect was observed at concentrations of gramicidin A higher than 10 μg/ml. No pigment was produced when gramicidin A and polymyxin B were added either singly or simultaneously to NPC in the absence of the inducer amino acid. (Fig. 2).

In a previous publication[3] we suggested that polymyxin B enhances prodigiosin production by modification of the outer membrane. We postulated that this facilitates the passage of L-proline to the inner plasma membrane and hence into the cytoplasm. In our view gramicidin A acts in a similar way to enhance the entry of L-proline. At a higher concentration of gramicidin A disorganization of the cytoplasmic membrane may occur to such extent that physiological as well as structural conditions for the biosynthesis of prodigiosin are severely altered.

On the other hand valinomycin (a K^+ ionophore) enhanced the synthesis of prodigiosin in NPC in a similar way as that observed with gramicidin A (Fig. 3). Maximum prodigiosin production could be reached in a valinomycin-containing medium in which 0.1 M KCl replaced 0.1 M NaCl. According to VIÑAS *et al.*[9] prodigiosin is linked to a protein that is present on the external face of the cytoplasmic membrane of *Serratia* and that its synthesis probably occurs in the surface of the inner membrane. By contrast, TSANG and KALLVY[10] suggested that prodigiosin biosynthesis takes place in the cytoplasm or in the inner membrane, and is associated with the bacterial cell wall. The effect of gramicidin A on prodigiosin production by NPC could be explained by the modifications of the bacterial envelope.

The amount of prodigiosin produced by NPC of *Serratia* is dependent on the levels of L-proline[1]. Our results demonstrate that, in the presence of gramicidin A or valinomycin, prodigiosin production is higher, probably due to an increase in the transport of L-proline. In the presence of potassium and valinomycin an enhancement in the chromogenesis could also be observed. These results suggest that K^+ transport, L-proline uptake and prodigiosin biosynthesis are interrelated. Despite its antimicrobial activity, the chemical structure of prodigiosin (linear tripyrrole) suggests that it could be related with energy metabolism in *Serratia*. These ideas could be the basis for further work about the actual role of prodigiosin in bacterial metabolism and the relationship between prodigiosin biosynthesis and the energetic state of the membrane.

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