INHIBITION BY STREPTOMYCIN OF THE BIOSYNTHESIS OF PRODIGIOSIN[1]

Robert P. Williams and Cora Lee Gott

Department of Microbiology

Baylor University College of Medicine

Houston, Texas

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The red pigment, prodigiosin, is produced typically by Serratia marcescens and also by other species of microorganisms (Lewis and Corpe, 1964). Wasserman et al. (1960b) and Rapoport and Holden (1960) formulated the structure of the pigment as a linear tripyrrole. Using mutants, Wasserman et al. (1960a) demonstrated that the terminal step in biosynthesis involves coupling of a stable bipyrrole, 4-methoxy-2, 2'-bipyrrole-5-carboxaldehyde, with a volatile monopyrrole, 2-methyl-3-amylpyrrole.

Various antibiotics prevented pigmentation without inhibiting growth of S. marcescens (Weil, 1952; Gott and Williams, 1963). Low concentrations of streptomycin inhibited the induced formation of pigment in nonproliferating suspensions (Blizzard and Peterson, 1963). We established in qualitative experiments that various antibiotics, including streptomycin, inhibited production of the bipyrrole moiety, but that synthesis of the monopyrrole and coupling of the two to form pigment were apparently not affected (Gott and Williams, 1963). Quantitative data, presented in this report, demonstrated that although streptomycin inhibited pigmentation primarily by affecting biosynthesis of the bipyrrole, production of the monopyrrole was also influenced.

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Strains of S. marcescens used were the wild-type strain Nima; a white mutant, strain 933, that produces the bipyrrole (kindly supplied to us by Dr. M. I. Bunting of Radcliffe College); and another white mutant, strain WF, that produces the monopyrrole. When 933 and WF were grown or mixed together, prodigiosin was produced in the mixed culture. Organisms were grown as broth cultures in the complete medium of Williams et al. (1956) minus the phosphate salts. Phosphate inhibited production of bipyrrole by mutant 933. Omission of phosphate removed this inhibition but also caused formation of a purple pigment after incubation for 3 d. This pigment did not have the solubility characteristics of prodigiosin. Assays for bipyrrole were carried out after incubation for 24 hr before the purple pigment accumulated.

Cultures were incubated at 27 C and analyzed for protein and pigment after 3 d. Protein was determined by the procedure of Lowry et al. (1951) using bovine albumin as a standard. Pigment in Nima or 933 was determined by adding 1.0 ml of a broth culture to 2.0 ml of 1 M KOH. The mixture was digested for 10 min in a bath of boiling H_2O. After digestion, the material was diluted to 0.1 its volume with water and acidified by the addition of 1.0 ml of 1 N HCl. The amount of pigment was determined by reading the optical density of this solution at 537 mu and subtracting from this reading the optical density at 655 mu to correct for absorption of nonspecific material. Determinations by this method agreed with those obtained by extracting pigment with acetone (Williams et al., 1956). Bipyrrole was measured by the method of Santer (1958) in which 1.0 ml of culture is shaken with 6.0 ml of $CHCl_3$. The amount of bipyrrole was then determined by reading the optical density of the chloroform extract at 363 mu and subtracting from this reading the optical density at 400 mu.

No reliable assay was developed for the volatile monopyrrole. Therefore, the amount of monopyrrole produced by WF was measured indirectly by determining the amount of pigment produced in 933. Strain

933 was grown for 48 hr in a broth medium containing 1.0% peptone and 5.0% glycerol in distilled water. A standard 1.0 ml suspension of these cells was smeared on the surface of peptone-glycerol agar in the cover of a Petri dish. Strain WF was grown in the bottom of the dish in complete medium minus phosphate salts. After incubation at 27 C for 3 d, the growth of 933 was scraped from the agar and the amount of pigment determined by the method given above.

The competency of WF to react with bipyrrole was determined by adding the chemically purified bipyrrole compound (kindly furnished by Dr. H. H. Wasserman of Yale University) directly to cultures of strain WF incubated for 24 hr. Competency of 933 to react with monopyrrole (Wasserman et al., 1960a) was determined by reacting cultures incubated for 3 d with 2,4-dimethylpyrrole (K & K Laboratories, Plainview, N. Y.). In both cases prodigiosin formed in control cultures immediately and was maximum within 5 to 10 min.

Figure 1 shows that low concentrations of streptomycin markedly inhibit formation of pigment and of bipyrrole. A concentration of 2.5 ug/ml inhibits about 95% of the production of pigment by strain Nima. Pigmentation is completely inhibited at a concentration of 10 ug/ml. Biosynthesis of pyrrole is not inhibited as rapidly as pigment, but again at a concentration of 10 ug/ml no bipyrrole can be detected. Except for the lag in inhibition, the two curves are similar. Concentrations of 10 ug/ml of streptomycin inhibit total growth (as determined by protein) of both organisms by about 35%. Growth of Nima ceases at concentrations of streptomycin between 25 and 50 ug/ml; growth of 933, between 50 and 100 ug/ml.

Since pigment formation in 933 depends upon the presence of the monopyrrole, it seems reasonable to assume that if a standard amount of bipyrrole is available, the amount of pigment formed in 933 will be a reflection of the quantity of monopyrrole provided. This assumption is the basis for the data shown in Fig. 2. When strain WF is

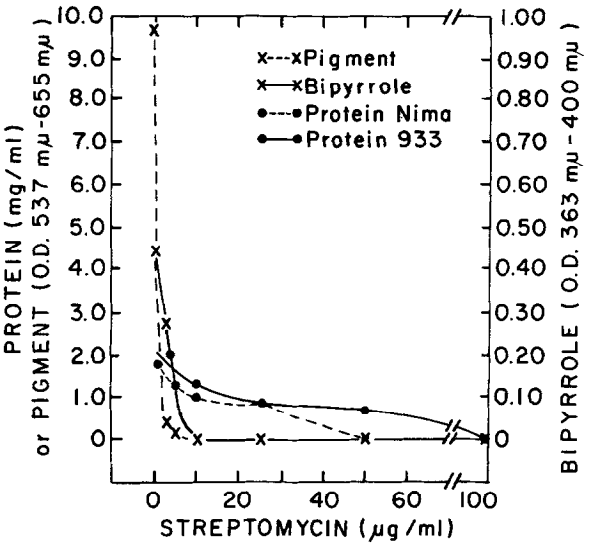

Figure 1. The effect of various concentrations of streptomycin upon growth and biosynthesis of pigment in S. marcescens strain Nima and of growth and biosynthesis of the bipyrrole in S. marcescens strain 933.

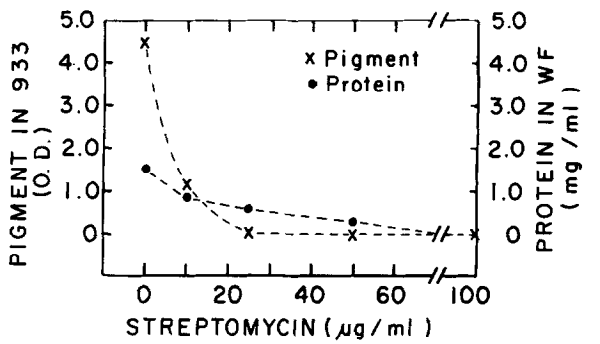

Figure 2. Effect of various concentrations of streptomycin upon growth of S. marcescens strain WF and biosynthesis of the monopyrrole by this strain. Production of the latter compound was measured indirectly by determining the amount of pigment formed in strain 933 by reaction with the monopyrrole synthesized in cultures of WF suspended in streptomycin.

grown in the presence of streptomycin, the amount of pigment formed in 933 is reduced. At a concentration of 10 ug/ml, indirect pigmentation in 933 is reduced about 75%. Complete inhibition of indirect pigmenta-

tion in 933 occurs at concentrations of streptomycin between 10 and 25 ug/ml. Thus biosynthesis of the monopyrrole is more resistant to inhibition by streptomycin than is that of the bipyrrole. Again, a concentration of 10 ug/ml of streptomycin reduces growth of strain WF by about 35%. Growth ceases at a concentration between 50 and 100 ug/ml.

TABLE I

Effect of Streptomycin upon Formation of Prodigiosin by Cultures of Serratia marcescens

Exper. No.	Cultures	Concentration of streptomycin (ug/ml)						
		0	5	10	25	50	100	10,000
1.	WF + bipyrrole	4+	4+	4+	3+	1+	0	-
2.	933 + monopyrrole	3+	1+	0	0	0	0	-
3.	WF + 933	4+	4+	4+	4+	4+	4+	4+

Amount of pigment was determined visually; 4+ indicates maximal pigmentation; 0, no pigment.

Cultures were incubated at 27 C (WF for 1 d; 933 for 3 d) in complete medium minus phosphate salts. In experiments 1 and 2, standard sized filter paper discs were saturated with the bipyrrole or monopyrrole, and the discs added directly to the cultures that were growing in the indicated concentration of streptomycin. In experiment 3, WF and 933 were grown separately without streptomycin. After incubation the indicated concentration of streptomycin was added to each culture and the suspensions reacted for 30 min. The two cultures were then mixed and observed for formation of pigment. All reactions were carried out at 27 C.

Table I shows that indirect measurement of the amount of monopyrrole produced by WF is not a sensitive determination. If the isolated bipyrrole is added to cultures of WF, formation of prodigiosin can be detected at concentrations of 50 ug/ml of streptomycin. Growth of WF still occurs at this concentration of antibiotic (Fig. 2). Thus some production of monopyrrole occurs even at high concentrations of strep-

tomycin that markedly inhibit growth of strain WF. In contrast, as shown in Table I, qualitative results obtained for the effect of streptomycin on production of the bipyrrole agree with the quantitative data. Table I also shows that streptomycin has no effect upon coupling of the monopyrrole with the bipyrrole to form prodigiosin.

These data indicate that streptomycin inhibits formation of prodigiosin by a growing culture of S. marcescens. Primarily, the drug inhibits biosynthesis of the bipyrrole. Inhibition of this compound parallels inhibition of pigmentation. Streptomycin also interferes with biosynthesis of the monopyrrole, although this compound still can be detected in cultures growing in high concentrations of streptomycin. However, since much lower concentrations of antibiotic inhibit formation of bipyrrole, reduction in synthesis of monopyrrole is of secondary importance in inhibition of pigmentation by growing cultures. Streptomycin does not interfere with coupling of the two compounds to form prodigiosin.

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