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Thiamine-induced Formation of the Monopyrrole Moiety of Prodigiosin

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Thiamine stimulates the production of a red pigment, which is chromatographically and spectrophotometrically identical to prodigiosin, by growing cultures of *Serratia marcescens* mutant 9-3-3. This mutant is blocked in the formation of 2-methyl-3-amylpyrrole (MAP), the monopyrrole moiety of prodigiosin, but accumulates 4-methoxy-2,2,'-bipyrrole-5-carboxaldehyde (MBC) and can couple this compound with MAP to form prodigiosin. Addition of thiamine caused production of MAP, and as little as 0.02 mg of thiamine per ml in a peptone-glycerol medium stimulated production of measurable amounts of prodigiosin. Phosphate salts and another type of peptone decreased the thiamine-induced formation of prodigiosin; yeast extract and glycerol enhanced the formation of this substance. Thiamine also enhanced production of prodigiosin by wild-type strain Nima of *S. marcescens*. The thiamine antagonists, oxythiamine and pyrithiamine, inhibited thiamine-induced production of MAP and of prodigiosin by the mutant strain 9-3-3, formation of prodigiosin by the wild-type strain Nima, and production of MAP by another mutant, strain WF. The pyrimidine moiety of thiamine was only 10% as effective as the vitamin; the thiazole moiety, only 4%; and the two moieties together, 25%. Various other vitamins tested did not stimulate formation of prodigiosin by strain 9-3-3. Thiamine did not stimulate production of prodigiosin by a single-step mutant that showed the same phenotypic block in prodigiosin biosynthesis as strain 9-3-3. This is not surprising since strain 9-3-3 originated as a result of two mutational events. One event may involve thiamine directly, and the other may involve the biosynthesis of MAP. Thiamine is probably involved in the regulation of the biosynthesis of MAP, because the vitamin or inhibitory antagonists must be added during the early phases of growth in order to be effective.

When grown aerobically at temperatures below 37 C, *Serratia marcescens* usually produces a red pigment, prodigiosin. The terminal step in the biosynthesis of prodigiosin (Fig. 1) involves the coupling of a stable bipyrrole, 4-methoxy-2,2',-bipyrrole-5-carboxaldehyde (MBC), with a volatile monopyrrole, 2-methyl-3-amylpyrrole (MAP), to form a linear tripyrrole (9, 15, 21).

Mutant strain 9-3-3 (13) is blocked in synthesis of MAP but can synthesize MBC (11, 12, 16) and the enzyme that couples these two moieties to form prodigiosin (9, 15, 19). If furnished with MAP or certain synthetic monopyrroles, strain 9-3-3 forms either prodigiosin or prodigiosin analogues (10, 15, 18, 21).

As reported in this paper, when thiamine is added to the growth medium, strain 9-3-3 produces a red pigment that has been identified as prodigiosin. The vitamin induces formation of MAP. Thiamine antagonists inhibit formation of prodigiosin and MAP in strain 9-3-3, synthesis of MAP in mutant strain WF, and production of prodigiosin by the wild-type strain Nima of *S. marcescens*.

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MATERIALS AND METHODS

Organisms. Strains of *S. marcescens* used in this investigation were (i) Nima, a typical, wild-type strain that produces prodigiosin (20); (ii) mutant WF (4, 21), which produces MAP but is blocked in synthesis of MBC, and yet can couple these two moieties to form prodigiosin (9, 21); (iii) mutant 9-3-3 (13), which excretes MBC and also produces the enzyme for terminal biosynthesis of prodigiosin, but is blocked in synthesis of MAP (15); and (iv) mutant H-262, which is phenotypically like strain 9-3-3. Mutant H-262 was isolated after a single exposure of the wild-type strain Hy of *S. marcescens* to ultraviolet irradiation.

Media. The organisms were maintained in stock on slants containing 2.5% agar, 0.5% peptone (Difco),

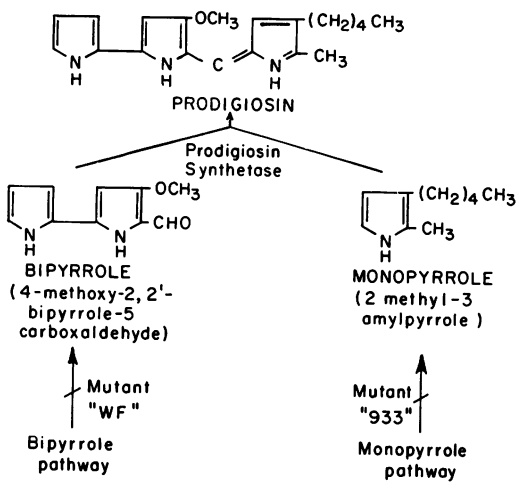

FIG. 1. *Pathway of prodigiosin biosynthesis.*

and either 0.5% L-proline or 1.0% glycerol. Liquid media used for quantitative experiments were (i) PG, a medium containing 0.5% peptone and 1.0% glycerol in distilled water (2); (ii) MM, the minimal medium of Bunting (2); (iii) CM, a complete medium (20) made by adding 0.1% yeast extract (Difco) and 0.2% N-Z Case peptone (Sheffield Chemical, Norwich, N.Y.) to MM medium; and (iv) SM, a synthetic medium (5) containing, in distilled water, 0.7% K_2HPO_4, 0.3% KH_2PO_4, 0.05% $Na_3C_3H_5O_7 \cdot 2H_2O$, 0.01% $MgSO_4$, 0.1% $(NH_4)_2SO_4$, and 0.2% glucose that was autoclaved separately and added aseptically to the other ingredients after they had been autoclaved. All media were adjusted to *p*H 7.2 before autoclaving. Thiamine and other test compounds were sterilized by filtration through membrane filters (Millipore Corp., Bedford, Mass.) and then added to various media as indicated in the text. Cultures were grown in 250-ml or 1-liter Erlenmeyer flasks containing 30 or 200 ml of liquid media, respectively.

Incubation. All cultures were incubated aerobically at 27 C. Liquid cultures were shaken on a New Brunswick rotary shaker (model G26) having a displacement of 1 inch and rotating at 198 strokes/min.

Pigment intermediates and chemicals. The supernatant fluid from cultures of strain 9-3-3 grown for 24 hr in PG medium was used as the source of MBC. Cells were removed by centrifugation, and the supernatant fluid was sterilized by membrane filtration (Millipore Corp.). Cultures of strain WF growing on PG agar were the source of the natural monopyrrole, MAP. An analogue of MAP, 2,4-dimethyl-3-ethylpyrrole (DEP), was purchased from Aldrich Chemical Co., Milwaukee, Wis. Thiamine·HCl, oxythiamine·HCl, the various other vitamins, and 2-methyl-4-amino-5-aminomethyl pyrimidine·HCl were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Pyrithiamine·HBr and 4-methyl-5-(β-hydroxyethyl)-thiazole were kindly provided by David Hendlin and H. Boyd Woodruff of the Merck Sharp and Dohme Research Laboratories, Rahway, N.J. The commercial compounds were diluted in 0.05 M phosphate buffer, *p*H 6.8, before sterilization.

Analytical procedures. Protein was determined by the method of Lowry et al. (7) with bovine serum albumin as a standard. The amount of MBC present was determined by first extracting the supernatant fluid with chloroform and then determining spectrophotometrically the difference in absorption at 363 and 400 nm (17). This difference was proportional to the amount of MBC in the supernatant fluid. In our experiments, a difference in absorbancy of 1.0 was equivalent to 28.6 μg of MBC per mg of bacterial protein. Prodigiosin and prodigiosin analogue were measured by extracting pigmented suspensions with acidic methanol (4.0 ml of 1 N HCl-96.0 ml of methanol) and then measuring the difference in absorption of the extract at 534 and 655 nm. Since the visible absorption spectra of prodigiosin and the analogue are almost identical (R. H. Williams, Ph.D. Thesis, Iowa State University, Ames, 1965), the same wavelength could be used to measure both compounds. A difference in absorbancy of 1.0 between the two wavelengths was equivalent to 19.3 μg of prodigiosin or 17.2 μg of prodigiosin analogue per mg of bacterial protein.

An indirect assay (19) was used to measure the volatile MAP because as yet no direct assay has been developed for this compound. The indirect assay utilized the syntrophic interaction of mutant strains WF and 9-3-3; the volatile MAP produced by strain WF, or by strain 9-3-3 when grown with thiamine, is coupled with the MBC synthesized by strain 9-3-3 to form prodigiosin. Strain WF or strain 9-3-3 was grown on the appropriate test medium in the bottom of a petri dish, and strain 9-3-3 was spread uniformly over the surface of PG agar in the top of the same petri dish. The amount of prodigiosin formed in the latter culture of strain 9-3-3, after incubation of the closed petri dish at 27 C for 48 hr, was then measured by the procedure given above. Since one molecule each of MBC and MAP couple to form one molecule of prodigiosin, the amount of pigment formed reflected the amount of volatile MAP produced, assuming an excess of MBC was available. The presence of this latter condition in the assay was established by preliminary experiments.

The following procedure was used to measure the ability of cells to couple MBC with DEP to form prodigiosin analogue. After centrifugation, the cells to be tested were resuspended in sterile supernatant fluid from cultures of strain 9-3-3 to a concentration of 0.5 to 1.0 mg of protein per ml. A 5-ml amount of this suspension was placed in a petri dish, and 250 μg of DEP was added. DEP was dissolved in about 2% ethyl alcohol in 0.5 M phosphate buffer, *p*H 6.8. The amount of analogue present in the mixture after incubation for 1 hr at 27 C was determined by the method described above.

RESULTS

When thiamine was added to PG medium, mutant strain 9-3-3 produced a red pigment (Fig. 2). Measurable amounts of pigment appeared at a thiamine concentration of 0.02 μg per ml of medium. Up to a concentration of 10 to 12 μg of

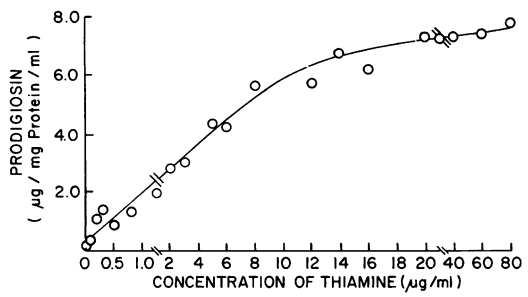

FIG. 2. *Effect of concentration of thiamine upon production of prodigiosin by S. marcescens strain 9-3-3. Thiamine was added at zero-hour. Cells were grown in PG medium and were harvested after growth for 24 hr at 27 C.*

TABLE 1. *Production of prodigiosin by S. marcescens strain 9-3-3 when grown on various liquid media*[a]

Medium	Prodigiosin (μg per mg of protein per ml)
0.5% peptone and 0.5% proline	0.21
0.5% peptone and 0.5% proline plus thiamine[b]	13.37
0.5% peptone and 1.0% glycerol	0.37
0.5% peptone and 1.0% glycerol plus thiamine[b]	17.93
Minimal medium (MM)	0.12
MM plus thiamine[b]	2.62
Complete medium (CM)	0.15
CM plus thiamine[b]	2.30
CM with phosphate salts omitted	1.24
CM with phosphate salts omitted plus thiamine[b]	48.37
Synthetic medium (SM)	0.23
SM plus thiamine[b]	1.91
SM with low phosphate[c] plus 0.1% tris-(hydroxymethyl)aminomethane (Tris) buffer	0.23
SM with low phosphate[c] plus 0.1% Tris buffer plus thiamine[b]	4.38

[a] All cultures were incubated with shaking at 27 C for 24 hr before assay.

[b] Thiamine added at a concentration of 100 μg/ml.

[c] Phosphate concentration reduced from 1.0% to 0.009%.

thiamine per ml, the amount of pigment formed increased almost linearly (Fig. 2). Higher concentrations of the vitamin resulted in only slight increases in pigmentation. Pigment extracted from these cultures was identical to an authentic sample of prodigiosin both in the visible absorption spectrum and in chromatographic behavior by paper, thin-layer, or column methods; these data indicated that the pigment was prodigiosin. As will be shown later, addition of thiamine to PG medium also enhanced production of prodigiosin by growing cultures of the wild-type strain Nima (Fig. 6).

TABLE 2. *Effect of complex natural nutrients upon thiamine-induced formation of prodigiosin by S. marcescens strain 9-3-3*[a]

Additions to basal medium[b]	Prodigiosin (μg per mg of protein per ml)
0.2% yeast extract (Difco)	1.35
0.2% yeast extract plus thiamine[c]	6.56
0.1% yeast extract	1.43
0.1% yeast extract plus thiamine[c]	26.11
0.1% yeast extract plus 0.2% N-Z Case peptone	1.25
0.1% yeast extract plus 0.2% N-Z Case peptone plus thiamine[c]	5.92
0.1% yeast extract plus 0.2% N-Z Case peptone plus 1% glycerol with thiamine[c]	1.40
0.2% N-Z Case peptone	0
0.2% N-Z Case peptone plus thiamine[c]	0.42
0.1% N-Z Case peptone	0
0.1% N-Z Case peptone plus thiamine[c]	0

[a] Cultural conditions were identical to those described in Table 1.

[b] Basal medium contained 0.5% dibasic ammonium citrate, 0.005% ferric ammonium citrate, 0.05% $MgSO_4$, 0.5% NaCl, and, with the exception noted, 0.2% glycerol in distilled water at *p*H 7.2. All media were sterilized by autoclaving.

[c] Thiamine was added at a concentration of 50 μg/ml.

Addition of thiamine to media other than PG also promoted formation of prodigiosin by strain 9-3-3 (Tables 1 and 2). Phosphate salts (Table 1) and N-Z Case peptone (Table 2) reduced the amount of prodigiosin formed even in the presence of thiamine. On the other hand, yeast extract and glycerol enhanced the effects of thiamine (Table 2; Fig. 3). In a medium containing 100 μg of thiamine per ml, 1.5% glycerol, and 0.2% yeast extract, almost 90 μg of prodigiosin per mg of protein was formed, a value that represented about 1 to 2% of the dry weight of cells. The details of these variations in composition of media were not investigated further. Since addition of thiamine either to PG medium or to CM medium from which phosphate salts were omitted induced formation of significant amounts of prodigiosin, these media were used in most of our experiments. Prodigiosin was not synthesized by strain 9-3-3 when biotin, niacin, pantothenate, *p*-aminobenzoate, pyridoxamine, riboflavine, or ascorbic acid was added to the growth medium in place of thiamine.

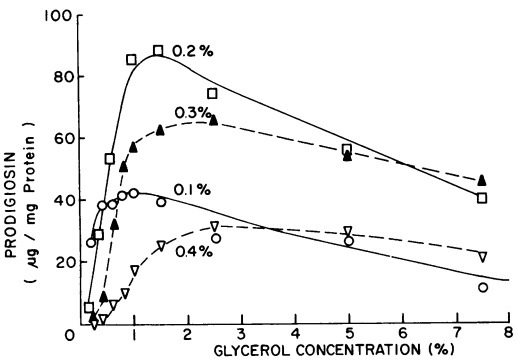

FIG. 3. *Effect of varying the concentration of yeast extract and of glycerol on thiamine-induced pigmentation by S. marcescens strain 9-3-3. Thiamine (100 µg/ml) was added at zero-hour to MM medium from which phosphate salts were omitted. This medium was supplemented with the concentrations of glycerol indicated on the abscissa, and with the concentrations of yeast extract (Difco) shown beside the individual curves. All cultures were incubated with shaking at 27 C for 24 hr before assay.*

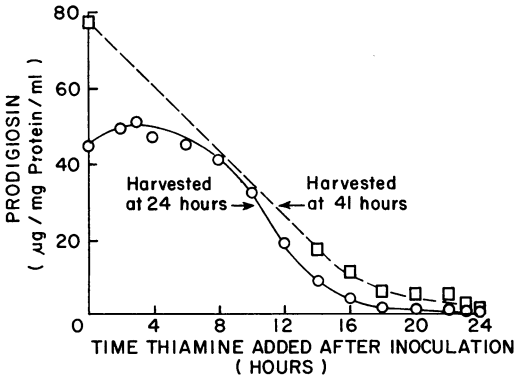

FIG. 4. *Effect of time of addition of thiamine on prodigiosin production by S. marcescens strain 9-3-3. Cultures were grown on a shaker at 27 C in CM medium from which phosphate salts were omitted. The thiamine concentration was 100 µg/ml. After harvesting the cultures at the indicated times, assays were performed.*

For effective induction of prodigiosin formation, thiamine must be added to the medium during the early stages of growth (Fig. 4). The later the addition of the vitamin once growth had been initiated, the less effect it had upon pigmentation. Thiamine added after 16 hr of incubation showed no effect when the cultures were assayed after 24 hr of incubation. Since some cultures were sampled 8 hr or less after the addition of the vitamin, the possibility existed that the interval between the addition of thiamine and sampling was too short for the detection of any effect on pigmentation. This possibility was evaluated by assaying again, at 41 hr, cultures to which thiamine was added after 12 hr of growth. The additional period of incubation produced no significant difference in the amount of pigment formed when compared to the 24-hr samples (Fig. 4).

Oxythiamine, an antagonist of thiamine, inhibited the effect of thiamine upon formation of prodigiosin by strain 9-3-3 (Fig. 5). At a molar ratio of 100:1 (oxythiamine to thiamine), prodigiosin formation was inhibited by about 66%. This process was more easily inhibited than growth, production of bipyrrole, or the ability of strain 9-3-3 to couple MBC with DEP to form prodigiosin analogue. At higher molar ratios, however, these metabolic activities were also inhibited. The ability of oxythiamine to reverse the effect of thiamine was only apparent when the antagonist was added during the first 10 hr of growth. This fact correlated with the observation that thiamine must be added during the early phases of growth to induce formation of prodigiosin.

Oxythiamine also inhibited the production of prodigiosin by the wild-type strain Nima (Figs. 6

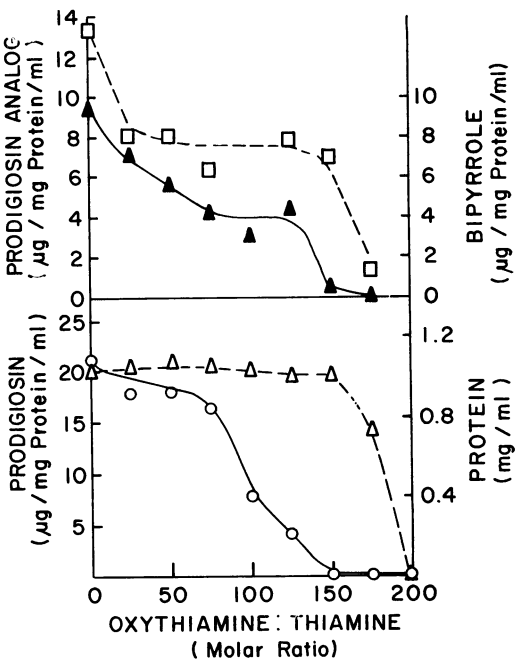

FIG. 5. *Effect of addition of oxythiamine to cultures of S. marcescens strain 9-3-3. Symbols: △, growth, as cellular protein; ○, thiamine-induced prodigiosin formation; ▲, bipyrrole (MBC) production; and □, the ability to couple MBC with DEP to form prodigiosin analogue. Cultures were assayed after growth on the shaker for 24 hr at 27 C. Oxythiamine and thiamine (10 µg/ml) were added to PG medium at zero-hour.*

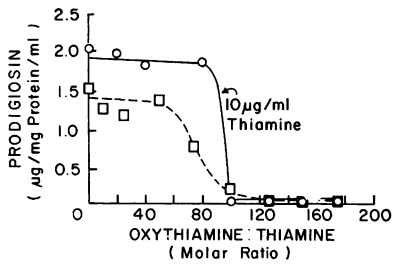

FIG. 6. *Effect of oxythiamine upon production of prodigiosin by wild-type strain Nima of S. marcescens grown in PG medium. Culture and assay conditions were the same as described in Fig. 5. Thiamine (10 μg/ml) was added to one set of cultures (○), and no vitamin was added to the other set (□). Note that the addition of only thiamine to PG medium enhanced the production of prodigiosin by about 5 μg per mg of protein per ml.*

and 7). The addition of a small amount of thiamine to PG medium (Fig. 6) exerted a protective effect upon inhibition of pigment formation by oxythiamine. The antagonist also inhibited formation of pigment by strain Nima and the formation of thiamine-induced pigment by strain 9-3-3 when these strains were grown in CM medium without phosphate (Fig. 7). Since CM medium contains some thiamine in the yeast extract [according to the manufacturer, dehydrated yeast extract (Difco) contains about 3 μg of thiamine per g], higher concentrations of oxythiamine were required for levels of inhibition comparable to those obtained in PG medium (Fig. 6). The concentration of oxythiamine shown in Fig. 7 was expressed as milligrams per milliliter rather than as a molar

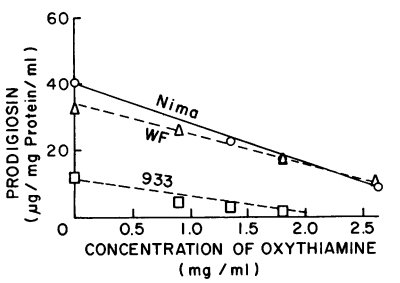

FIG. 7. *Effect of oxythiamine upon production of prodigiosin by strains Nima (○) and 9-3-3 (□) of S. marcescens, grown in CM medium from which phosphate salts were omitted, and upon the production of MAP by strain WF (△) grown on PG agar. Thiamine (2 μg/ml) was added to cultures of strain 9-3-3. In these experiments, 1.8 mg of oxythiamine per ml of CM medium is equivalent to a molar ratio of 500:1 of thiamine in PG medium. Cultures of strains Nima and 9-3-3 were assayed after growth on a shaker for 24 hr at 27 C. MAP production by strain WF was measured by the indirect assay.*

ratio because the exact amount of thiamine in the medium was unknown. In addition, production of MAP was inhibited when mutant strain WF was grown on PG medium containing oxythiamine (Fig. 7).

The induction of prodigiosin formation in strain 9-3-3 by thiamine was very sensitive to another antagonist, pyrithiamine. A molar ratio of antagonist to vitamin of 10:1 inhibited formation of prodigiosin by about 95%, while reducing growth only about 25%; the production of MBC was not markedly reduced, nor was the ability to couple MBC with DEP to form prodigiosin analogue (Fig. 8).

The pyrimidine moiety of thiamine, 2-methyl-4-amino-5-aminomethyl pyrimidine, was about 10% as effective as the vitamin in inducing prodigiosin formation in strain 9-3-3 (Table 3). The thiazole moiety, 4-methyl-5(β-hydroxyethyl)-thiazole, was even less effective. Addition of both moieties to PG medium gave about 25% of the response of thiamine itself.

The above data suggested that the addition of thiamine enabled strain 9-3-3 to synthesize MAP.

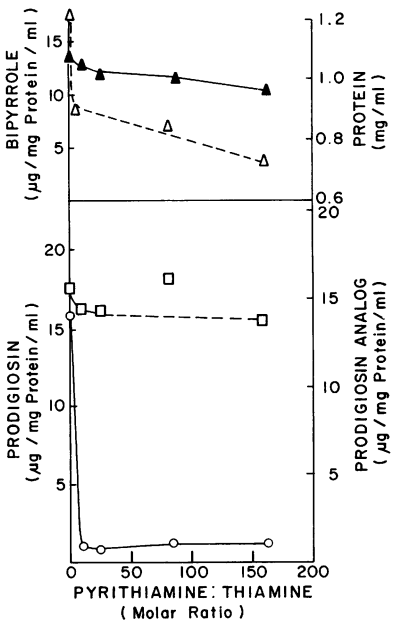

FIG. 8. *Effect of addition of pyrithiamine to cultures of S. marcescens strain 9-3-3. Symbols: △, growth, as cellular protein; ▲, bipyrrole (MBC) production; ○, thiamine-induced prodigiosin formation; and □, the ability to couple MBC with DEP to form prodigiosin analogue. Cultures were assayed after growth on a shaker for 24 hr at 27 C. Pyrithiamine and thiamine (2 μg/ml) were added at zero-hour to PG medium.*

TABLE 3. *Induction of prodigiosin synthesis in S. marcescens strain 9-3-3 by thiamine and its moieties*[a]

Compound[b] added to PG medium	Prodigiosin formed	
	Amt per mg of protein per ml	Per cent formed (compared to thiamine)
	μg	
None	0.10	1.1
Thiamine·HCl	9.42	100.0
Pyrimidine moiety[c]	1.10	11.7
Thiazole moiety[d]	0.37	3.9
Pyrimidine and thiazole moieties	2.18	23.1

[a] All cultures were incubated with shaking at 27 C for 24 hr before assay.

[b] All compounds were added at a concentration of 100 μg/ml.

[c] 2-Methyl-4-amino-5-aminomethyl pyrimidine·HCl.

[d] 4-Methyl-5-(β-hydroxyethyl)-thiazole.

TABLE 4. *Production of volatile monopyrrole (MAP) by S. marcescens strain 9-3-3*[a]

Medium in bottom of petri dish	MAP (as prodigiosin) formed (per mg of protein per ml) × 10^4	Increase over control
	μg	
Complete medium (CM) with phosphate salts omitted	9	
CM with phosphate salts omitted plus thiamine[b]	25	2.8 ×
Peptone-glycerol (PG)	9	
PG plus thiamine[b]	42	4.7 ×

[a] MAP was measured by the indirect assay utilizing syntrophic pigmentation. After incubation for 48 hr at 27 C, cultures of strain 9-3-3 growing on PG medium in the top of the dishes were harvested and assayed for protein and prodigiosin.

[b] Thiamine concentration was 50 μg/ml.

If this hypothesis were true, then any volatile MAP produced by strain 9-3-3 growing on medium enriched with thiamine would promote syntrophic pigmentation in other cultures of strain 9-3-3 growing in the absence of the vitamin. As can be seen in Table 4, more MAP was produced on medium enriched with thiamine than was required for formation of prodigiosin by strain 9-3-3, since cultures growing on media containing thiamine not only formed prodigiosin, but also promoted syntrophic formation of prodigiosin in cultures lacking the vitamin.

DISCUSSION

Thiamine induced formation of a red pigment in mutant strain 9-3-3 by stimulating production of MAP. Spectral and chromatographic evidence indicated that the pigment was prodigiosin. Thiamine did not cause reversion of strain 9-3-3 to a pigmented state, nor did it favor the selection of a spontaneous, pigmented mutant. When red-pigmented cells of strain 9-3-3 were harvested, washed, and then inoculated into fresh medium without thiamine, their ability to form prodigiosin disappeared.

Thiamine also enhanced production of prodigiosin by the wild-type strain Nima. However, strain H-262, a single-step mutant that is phenotypically like strain 9-3-3, did not form pigment when grown in the presence of thiamine. This was not surprising, since strain 9-3-3 is a spontaneous mutant isolated from another spontaneous mutant, strain 9-3, that originated from the wild-type strain 274 of *S. marcescens* (U. V. Santer, Ph.D. Thesis, Yale Univ., New Haven, Conn., 1958). Thus, strain 9-3-3 arose as the result of two mutations. Although both mutational events might affect biosynthesis of MAP, only one might involve thiamine. The vitamin might relieve one block, allowing synthesis of an intermediate that could be used to overcome the second block. Relief of the second block would permit biosynthesis of MAP.

Mutant strain 9-3-3 grew well on a minimal medium containing no thiamine. Therefore, the organism can synthesize enough of the vitamin to carry out basic metabolic reactions. The inhibitory effect upon growth of the thiamine antagonists oxythiamine and pyrithiamine supports this assumption. Mutant strain 9-3-3 also apparently synthesizes only a limited amount of the pyrimidine component of the vitamin, since additional amounts of this moiety do induce formation of some prodigiosin. Addition of the thiazole moiety induces formation of only a limited amount of pigment. Addition of both moieties together does not cause formation of pigment equivalent to that formed by the addition of the whole molecule; thus, the mutant evidently has a limited capacity to couple the two moieties to form thiamine.

Wild-type and mutant strains of *S. marcescens* possess catalase activity (22). Mutant strain 9-3-3 also contains this enzyme (*unpublished data*). In addition, strain 9-3-3 grows well aerobically, presumably by utilizing cytochrome enzymes. These observations indicate that the mutant synthesizes the pyrrole groups of the porphyrins found in these enzymes. The usual pathway for pyrrole biosynthesis involves δ-amino-levulinic

acid, but this compound is poorly incorporated into prodigiosin (8). This fact suggests that the pyrrole groups of prodigiosin are synthesized by a different pathway than the pyrroles of the porphyrins. The pyrrole moieties of prodigiosin, MAP and MBC, are probably synthesized by separate pathways (Fig. 1), although they may have common early precursors (9). Our data indicate that thiamine influences synthesis of MAP, but the effect of the vitamin may be indirect and may involve some other pathway, rather than affecting synthesis of MAP directly. The vitamin does not substitute for MAP because addition of thiamine to suspensions of cells of strain 9-3-3 produces no pigment. Since thiamine must be added early during growth for the most effective induction of prodigiosin biosynthesis, and corroboratively, antagonists of thiamine must also be added early to cause inhibition, the vitamin probably influences early steps in either the regulation or the biosynthesis of pigment.

Phosphate salts inhibit biosynthesis of prodigiosin by wild-type organisms (1, 3), biosynthesis of MBC in strain 9-3-3 (19), and biosynthesis of MAP and MBC in other mutants (D. A. Jackson, M. I. Bunting, and D. A. Morrison, Bacteriol. Proc. p. 53, 1963). Phosphate causes a similar inhibition of thiamine-induced pigmentation in strain 9-3-3. The effect of thiamine and of the phosphate salts may be related and may involve a common biosynthetic step. Interestingly, when strain 9-3-3 is grown in the absence of thiamine in media lacking phosphate salts (6) or in PG medium, it produces a purple pigment. This pigment has different spectral characteristics than prodigiosin and is probably identical to the dipyrrolyldipyrromethene analogue of prodigiosin recently reported (14).

An apparent paradox exists in that strain 9-3-3 can synthesize enough thiamine to carry out its basic metabolic functions, but not enough of the vitamin is available to permit synthesis of the nonessential compound, prodigiosin. Perhaps the effect of thiamine upon biosynthesis of MAP does not involve the vitamin in its usual role as a coenzyme. Nothing is yet known of the early biosynthetic steps leading to formation of prodigiosin, and these steps must be elucidated before the effect of thiamine can be explained.

ACKNOWLEDGMENT

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