Inhibition of *Vibrio harveyi* Bioluminescence by Cerulenin: In Vivo Evidence for Covalent Modification of the Reductase Enzyme Involved in Aldehyde Synthesis

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Bacterial bioluminescence is very sensitive to cerulenin, a fungal antibiotic which is known to inhibit fatty acid synthesis. When *Vibrio harveyi* cells pretreated with cerulenin were incubated with [^{3}H]myristic acid in vivo, acylation of the 57-kilodalton reductase subunit of the luminescence-specific fatty acid reductase complex was specifically inhibited. In contrast, in vitro acylation of both the synthetase and transferase subunits, as well as the activities of luciferase, transferase, and aldehyde dehydrogenase, were not adversely affected by cerulenin. Light emission of wild-type *V. harveyi* was 20-fold less sensitive to cerulenin at low concentrations (10 μg/ml) than that of the dark mutant strain M17, which requires exogenous myristic acid for luminescence because of a defective transferase subunit. The sensitivity of myristic acid-stimulated luminescence in the mutant strain M17 exceeded that of phospholipid synthesis from [^{14}C]acetate, whereas uptake and incorporation of exogenous [^{14}C]myristic acid into phospholipids was increased by cerulenin. The reductase subunit could be labeled by incubating M17 cells with [^{3}H]tetrahydrocerulenin; this labeling was prevented by preincubation with either unlabeled cerulenin or myristic acid. Labeling of the reductase subunit with [^{3}H]tetrahydrocerulenin was also noted in an aldehyde-stimulated mutant (A16) but not in wild-type cells or in another aldehyde-stimulated mutant (M42) in which [^{3}H]myristoyl turnover at the reductase subunit was found to be defective. These results indicate that (i) cerulenin specifically and covalently inhibits the reductase component of aldehyde synthesis, (ii) this enzyme is partially protected from cerulenin inhibition in the wild-type strain in vivo, and (iii) two dark mutants which exhibit similar luminescence phenotypes (mutants A16 and M42) are blocked at different stages of fatty acid reduction.

Bacterial luminescence results from the luciferase-catalyzed oxidation of a long-chain aliphatic aldehyde (myristyl aldehyde) and reduced flavin mononucleotide by molecular oxygen (for a review, see reference 16). The aldehyde substrate is synthesized by a luminescence-specific fatty acid reductase complex consisting of three subunits: an acyltransferase (T) responsible for supplying endogenous fatty acid from acyl-acyl carrier protein (ACP) (4, 8); a synthetase (S) which activates fatty acid to form acyl-S via an acyl-AMP intermediate (22, 25); and a reductase (R) which uses NADPH to reduce acyl-S (or acyl-coenzyme A [CoA]) to produce the corresponding aldehyde (23). The fatty acid reductase complex and luciferase are coordinately induced during the late-exponential growth phase characteristic of light emission in vivo (16).

Cerulenin [(2*S*)(3*R*)-2,3-epoxy-4-oxo-7,10-dodecadienoyl amide] is an antibiotic produced by *Cephalosporium caerulens*, which is known to inhibit fatty acid and sterol synthesis, fatty acylation of proteins, and other membrane-related processes (for a review, see reference 19). Apart from its effects on fatty acid synthesis, where it has been shown to covalently and irreversibly inhibit β-ketoacyl-ACP synthetase (9, 29), the site(s) and mechanism of cerulenin inhibition in the above processes are largely unknown. The sensitivity of bacterial bioluminescence to cerulenin was originally reported by Ulitzur and Goldberg (27) and used by

these authors to develop a bioassay for this compound. Grogan (11) demonstrated that inhibition of bioluminescence by cerulenin is not caused by its primary effect on fatty acid biosynthesis and suggested that fatty acid reduction to aldehyde could be the impaired step.

This investigation was undertaken to elucidate the enzymatic step(s) at which cerulenin inhibits bioluminescence in *Vibrio harveyi*, a species for which a number of dark mutants have been characterized. The results clearly demonstrate that cerulenin specifically and covalently blocks the transfer, reduction, or both, of fatty acids at the level of the R subunit in vivo and that the extent of this inhibition may be a function of the fatty acyl flux through the enzyme system.

MATERIALS AND METHODS

Materials. [9,10-^{3}H]myristic acid (30 Ci/mmol) was prepared by Amersham Canada Ltd. (Oakville, Ontario, Canada) by tritiation of the corresponding monounsaturated fatty acid. [7,8,10,11-^{3}H]tetrahydrocerulenin (150 Ci/mmol) was prepared in a similar manner from cerulenin by Du Pont Canada Ltd. (NEN Products, Lachine, Quebec, Canada) and was further purified by thin-layer chromatography before use (21). Sodium [1-^{14}C]acetate (58 Ci/mol), [1-^{14}C] myristic acid (60 Ci/mol), and En^{3}Hance were also purchased from Du Pont Canada Ltd. [^{3}H]myristoyl-CoA (23) and [^{3}H]myristoyl-ACP (8) were prepared as previously described. Myristic acid, cerulenin, and reagents and molecular weight standards for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were obtained from Sigma Chemical Co., St. Louis, Mo. Thin-layer chromatography plates (Silica Redi-Plate G) were obtained from Fisher

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Scientific Co., Dartmouth, Nova Scotia, Canada. All sol-
vents and other materials were reagent grade or better.
Stock solutions of fatty acids (stored at −20°C) or cerulenin
(prepared fresh) were stored in ethanol and were added to
cells so that the final solvent concentration was <1%.

Bacterial cultures. All strains used in this study, including
V. harveyi B392 and dark mutant strains M17 and A16 (30),
AFM (8), and M42 (28), have been described previously. *V.
harveyi* strains were grown at 27°C in complex medium (20)
containing 1% NaCl. Cell growth was monitored by A_{660},
where 1 A_{660} unit equals 5 × 10^8 cells per ml. Luminescence
was monitored with a photomultiplier photometer as previ-
ously described (30), where 1 light unit equals 5 × 10^9 quanta
per s. Luminescence of the M17 mutant was determined
after the addition of 100 μM myristic acid, while that of the
A16 and M42 mutants was measured in the presence of 100
μM decyl aldehyde.

Protein labeling and SDS-PAGE. Labeling of proteins in
vivo with [^{3}H]myristic acid and in vitro with [^{3}H]myristic
acid (+ATP) or [^{3}H]myristoyl-CoA was performed as out-
lined by Byers et al. (6). For in vivo labeling, portions of
culture (typically 1 ml) were incubated with [^{3}H]myristic
acid for 10 min under growth conditions. For in vitro
labeling, cell extracts (20 μl) were incubated for 10 min with
8 μM [^{3}H]myristoyl-CoA (1 Ci/mmol) in a total volume of 50
μl of 50 mM phosphate–10 mM β-mercaptoethanol (pH 7) or
with 8 μM [^{3}H]myristic acid (30 Ci/mmol) in the same buffer
containing 5 mM ATP and 10 mM MgSO$_4$. Protein samples
were prepared, analyzed by SDS-PAGE (15), and visualized
by fluorography as described previously (30). Labeled pro-
teins were identified by comparison to published profiles (8,
30) and from the migration of protein standards (in kilodal-
tons [kDa]): bovine serum albumin, 66; ovalbumin, 45;
glyceraldehyde-3-phosphate dehydrogenase, 36; carbonic
anhydrase, 29; trypsinogen, 24; soybean trypsin inhibitor,
20.1; α-lactalbumin, 14.2.

Lipid extraction and separation. Incubation of cells with
[^{14}C]acetate and [^{14}C]myristic acid and extraction of total
lipid by the method of Bligh and Dyer (1) were carried out
essentially as described previously (5). Total lipid extracts
were fractionated on thin-layer chromatography plates de-
veloped in chloroform-methanol-acetic acid-water (85:15:
10:3.5 [vol/vol/vol/vol]) and labeled phospholipids were
identified by using a Bioscan System 200 imaging detector
(5). The incorporation of labeled precursor was calculated
from the percentage of total radioactivity in the phosphati-
dylethanolamine, phosphatidylglycerol, and cardiolipin
bands and from the specific radioactivity of the added
precursor.

Enzyme assays. Cell lysis and enzyme assays were carried
out as described earlier (6). Briefly, cell-free extracts for
enzyme assays and in vitro fatty acid labeling studies were
prepared by sonication of cell pellets (from 20 ml of culture)
in 1 ml of 50 mM phosphate–10 mM β-mercaptoethanol (pH
7.0), followed by centrifugation (17,000 × g, 30 min). Lu-
ciferase activity was measured by monitoring luminescence
after the injection of catalytically-reduced flavin mononucle-
otide into solutions containing extract and decyl aldehyde
(12). Aldehyde dehydrogenase activity was determined spec-
trophotometrically (7), while transferase (acyl-ACP cleav-
age) activity was measured by liquid scintillation counting of
the hexane-soluble radioactivity after incubation with
[^{3}H]myristoyl-ACP (8). Details are provided in the Table 1
footnotes. Protein was measured by using the Bio-Rad
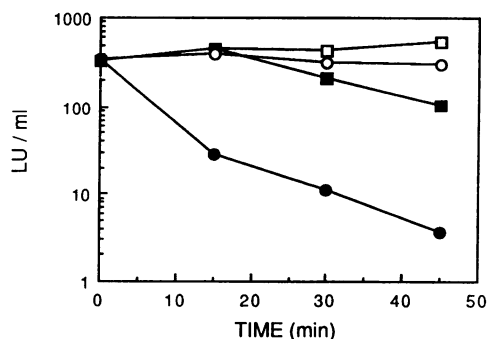Laboratories dye-binding assay (2), with bovine serum albu-
min used as a standard.

FIG. 1. Inhibition of *V. harveyi* wild-type and mutant M17 lumi-
nescence by cerulenin. Cultures of the wild type (□, ■) and M17 (○,
●) were grown to an A_{660} of 1.4 (350 light units [LU]/ml); 25-ml
samples were removed and further incubated with cerulenin at a
final concentration of 10 μg/ml (closed symbols) or with ethanol
alone (open symbols). At the times indicated, 1-ml portions were
removed for the measurement of luminescence as described in the
text.

RESULTS

Although previous investigations have examined the inhi-
bition of luminescence by cerulenin with both the wild-type
and mutant M17 strains of *V. harveyi* (11, 27), the relative
sensitivities of these strains to the antibiotic under condi-
tions that do not inhibit bacterial growth have not been
reported. In the experiment shown in Fig. 1, light emission
was monitored as a function of time after the addition of
cerulenin at a sublethal concentration: 10 μg/ml (27). Wild-
type luminescence was inhibited about fivefold over the
45-min incubation period, while the decrease in myristic
acid-stimulated luminescence of the M17 mutant was much
more dramatic (100-fold) over the same period (Fig. 1).
Addition of decyl aldehyde to either strain after cerulenin
inhibition immediately and completely restored light emis-
sion to control levels (data not shown), consistent with
previous observations that cerulenin does not adversely
affect either luciferase or the overall cellular metabolism
supporting bioluminescence (11, 27). This was also shown
directly by assaying the in vitro activities of luminescence-
related enzymes from cerulenin-treated cultures: no appre-
ciable inhibition of either luciferase, aldehyde dehydrogen-
ase, or T (acyl-ACP cleavage) was observed (Table 1).

The sensitivity of the M17 mutant to cerulenin raises the
possibility that myristic acid uptake (which presumably must
occur for the stimulation of light emission in this dark
mutant) is a preferential site of inhibition. This was ad-
dressed by measuring the incorporation of radiolabeled
precursors into phospholipids in parallel with luminescence
as a function of cerulenin concentration in the M17 mutant
(Fig. 2). Both luminescence and [^{14}C]acetate incorporation
into total phospholipid (i.e., reflecting acetate uptake, de
novo fatty acid synthesis, and phospholipid acylation) were
inhibited to a similar extent at low cerulenin concentrations
(50% inhibition within 30 min at 2 μg/ml), whereas inhibition
of luminescence was slightly greater at higher concentra-
tions. In marked contrast, incorporation of exogenous
[^{14}C]myristate was not inhibited but was increased (about
twofold) at low cerulenin concentrations. These results
clearly demonstrate that uptake of, or phospholipid acyla-
tion with, exogenous fatty acids is not adversely affected by
cerulenin (the latter may in fact be enhanced by the reduced
contribution of endogenous fatty acids under these condi-
tions; 5). Moreover, these data indicate that aldehyde syn-

TABLE 1. Effects of cerulenin on bioluminescence-related enzyme activities of V. harveyi wild type and mutant M17[a]

Presence of cerulenin in strains	Luciferase (LU/mg)[b]	Aldehyde dehydrogenase (μmol/min per mg)[c]	Transferase (cpm/min per μg)[d]
Wild type			
−	1,530	67	670
+	1,190	58	570
M17			
−	1,650	49	0
+	1,640	56	0

[a] Cultures (as described in the Fig. 1 legend) were preincubated for 45 min in the presence (+) or absence (−) of 10 μg of cerulenin per ml. Cell-free extracts (7 to 9 mg of protein per ml) were prepared and used to measure enzyme activities as described below and in the text. The mean of duplicate determinations is shown.

[b] Luciferase activity was determined by using 1 μl of extract and 60 μM decyl aldehyde in 1 ml of 50 mM phosphate (pH 7) containing 0.2% bovine serum albumin. LU, Light unit.

[c] Aldehyde dehydrogenase activity was measured with 10 μl of extract, 100 μM dodecyl aldehyde, and 1.5 mM NAD$^+$ in 50 mM phosphate–10 mM β-mercaptoethanol (pH 7).

[d] Transferase (acyl-ACP cleavage) activity was determined after the incubation of 1 μl of extract with [^{3}H]myristoyl-ACP (60,000 cpm) for 2 min in 1 M phosphate (pH 7).

thesis is at least as sensitive to cerulenin as fatty acid synthesis in the M17 mutant.

To determine the site(s) of aldehyde synthesis that might be directly affected by cerulenin, the effect of this agent on the acylation of luminescence-specific enzyme intermediates was examined. We have previously shown that, while fatty acid reductase activity cannot be detected in extracts of V. harveyi, the R, S, and T enzymes from this strain can be identified by labeling with [^{3}H]myristic acid, [^{3}H]myristoyl-CoA, or both (30). Preincubation of cells with cerulenin blocked acylation of the 57-kDa R subunit with [^{3}H]myristic acid in vivo but had little effect on the labeling of the 42-kDa S subunit with fatty acid either in vivo or in vitro (except in

FIG. 3. Effects of cerulenin on the fatty acylation of V. harveyi enzymes involved in aldehyde synthesis. Cultures of the wild type and M17 were preincubated for 45 min with (+) or without (−) cerulenin at 10 μg/ml (see the Fig. 1 legend). At this time, portions were removed for the labeling of proteins in vivo with [^{3}H]myristic acid (30 Ci/mmol; final concentration, 2 μM) (A) and cell extracts were prepared for the labeling of proteins in vitro with [^{3}H]myristic acid plus ATP (B) or with [^{3}H]myristoyl-CoA (C) as described in the text. Labeled proteins were separated by SDS-PAGE and visualized by fluorography.
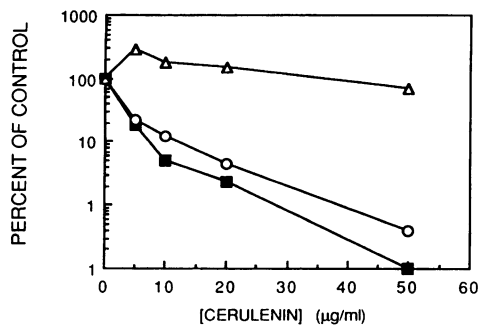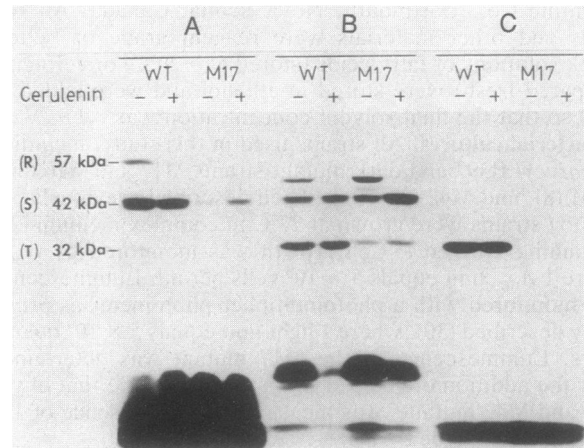

FIG. 2. Effects of cerulenin on phospholipid synthesis from endogenous and exogenous precursors in the V. harveyi M17 mutant. Portions (5 ml) from a culture of M17 grown to an A_{660} of 1.5 (450 light units per ml) were incubated with cerulenin at the concentrations indicated. After 30 min, 1-ml samples from each were removed for measurement of luminescence (■) and incorporation of [^{14}C]acetate (○) and [^{14}C]myristic acid (△) into phospholipid. Samples were incubated with [^{14}C]acetate (5 μCi; final concentration, 86 μM) or [^{14}C]myristic acid (0.12 μCi; final concentration, 50 μM) for 10 min, and total phospholipids were extracted and isolated as described in the text. Data are expressed as the percentage of control values in the absence of cerulenin: luminescence, 400 light units per ml; [^{14}C]acetate incorporation, 640 pmol/ml; and [^{14}C]myristic acid incorporation, 3,670 pmol/ml.

the M17 mutant, in which increased acylation of the S subunit was observed) (Fig. 3). In vitro labeling of the R subunit is variable (30) and was not observed in this experiment. Cerulenin did produce a decrease in labeling of the T enzyme in vivo; however, this was not observed with either [^{3}H]myristic acid or [^{3}H]myristoyl-CoA in vitro (Fig. 3), nor was there any effect on the activity of this enzyme (Table 1). This could be a secondary effect, since cerulenin also inhibited the labeling of the 20-kDa band identified as the acyl-ACP substrate of the T enzyme (7), as well as proteins in the 50-kDa range that are not luminescence specific (30). In any case, the present data suggest that the specific effect of cerulenin on the luminescence system is via inhibition of the reductase enzyme.

The above conclusion was supported by incubating M17 cells with the radiolabeled cerulenin analog [^{3}H]tetrahydrocerulenin, which has previously been used to detect fatty acid synthetase and related enzymes in cell extracts (21). Despite prior purification of [^{3}H]tetrahydrocerulenin by thin-layer chromatography, the compound was very unstable and many bands were invariably detected by SDS-PAGE and fluorography under our labeling conditions (Fig. 4). However, only a single protein of 57 kDa (i.e., corresponding in size to the R subunit) appeared to be specifically modified by the inhibitor (Fig. 4, lane 1), since its labeling was abolished by preincubation of cells with unlabeled cerulenin (lane 3) and was substantially reduced in the presence of myristic acid (lane 2). These results strongly indicate that the R subunit is specifically and covalently inhibited by cerulenin and suggest that myristic acid can partially protect the enzyme from this modification.

Labeling of the reductase subunit by [^{3}H]tetrahydrocerulenin in the V. harveyi wild type and dark mutants was found to be highly strain specific (Fig. 5). In contrast to the M17 mutant, little or no labeling of the R subunit was observed with the wild-type strain, which indicated a correlation between [^{3}H]tetrahydrocerulenin modification and sensitivity of light emission to cerulenin (Fig. 1). A similar
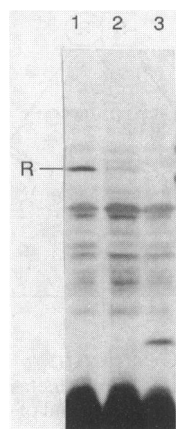
FIG. 4. Labeling of *V. harveyi* M17 cells in vivo with [³H] tetrahydrocerulenin and the effect of preincubation with cerulenin and myristic acid. Culture portions (1 ml) of the M17 mutant (grown to an A_{660} of 1.5) were incubated for 30 min with 0.5 mCi of [³H] tetrahydrocerulenin either directly (lane 1), after preincubation for 10 min with 100 μM myristic acid (lane 2), or after preincubation for 10 min with 20 μg of cerulenin per ml (lane 3). After labeling, cerulenin (20 μg) was added to all samples and cell pellets were used to prepare samples for SDS-PAGE and fluorography exactly as described for in vivo fatty acid labeling in the text. The migration position of the reductase subunit (R [57 kDa]) is shown.

FIG. 6. In vivo pulse-chase labeling of *V. harveyi* proteins with [³H]myristic acid. Culture portions (1 ml) of wild-type (1), M17 (2), A16 (3), and M42 (4) cells grown to an A_{660} of 1.7 to 2.3 were incubated with [³H]myristic acid (30 Ci/mmol; final concentration, 1.2 μM). After 10 min, 0.5 ml was removed for preparation of SDS-PAGE samples directly (P, pulse), the remaining sample was centrifuged briefly (12,000 × g, 3 min), and the pellet was suspended in 0.5 ml of growth medium containing 100 μM unlabeled myristic acid for a further incubation of 20 min (C, chase). A fluorogram of proteins separated by SDS-PAGE is shown; the migration positions of the reductase (R [57 kDa]), synthetase (S [42 kDa]), and transferase (T [32 kDa]) subunits are indicated.

result was obtained with the aldehyde-stimulated M42 mutant, whereas a mutant with an identical luminescence phenotype (A16) exhibited labeling of the R subunit. The AFM mutant provided a negative control in this experiment, since no bioluminescence functions are expressed in this strain (7). Minor variations in the nonspecific labeling of other proteins, caused by the different overall protein profiles among the pleiotropic dark mutants, can also be observed in Fig. 5. The above results indicate that the sensitivity of the reductase subunit to modification with cerulenin can vary with the activity or integrity of the enzymes
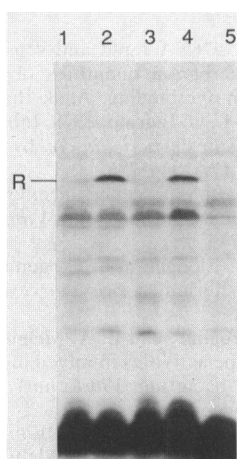
involved in aldehyde synthesis. Moreover, they suggest that the A16 and M42 mutants, both defective in aldehyde synthesis from fatty acid, are blocked at different stages of the fatty acid reductase pathway.

To further explore possible differences in the enzyme defects of aldehyde-deficient *V. harveyi* mutants, the turnover of fatty acyl-enzyme intermediates was investigated in an in vivo pulse-chase experiment with [³H]myristic acid. The [³H]myristoyl-enzyme intermediates of the S and R subunits could be chased effectively with unlabeled fatty acid in the wild-type strain as well as in the M17 mutant (in which a lower level of steady-state acylation was observed; 30) (Fig. 6). This evidence for catalytic turnover was expected, since both strains exhibit the ability to reduce fatty acid to aldehyde in vivo. As noted previously (30), neither the S nor R subunit was acylated by [³H]myristic acid in the A16 mutant, which is defective in aldehyde synthesis. In contrast, the S subunit was heavily pulse-labeled in the M42 mutant, while the reductase enzyme was acylated at a much lower level in comparison to the wild type (Fig. 6). Label associated with the S subunit was chased by unlabeled fatty acid, but no turnover of acyl groups on the R subunit was observed. These results confirm that the two aldehyde-stimulated dark mutants A16 and M42 are biochemically distinct, which further indicates that different molecular lesions of fatty acid reduction to aldehyde are involved.

DISCUSSION

Cerulenin is known to inhibit a variety of membrane-related processes, such as fatty acid, sterol, and polyketide synthesis (19), as well as protein acylation (14, 26) and antigen processing (10). In most cases, the primary site of cerulenin action in these processes has not been established. The exception is fatty acid synthesis, where covalent inhibition of the rate-limiting condensing enzyme, β-ketoacyl-ACP synthetase, has been demonstrated (9, 29). Specifically, cerulenin has been shown to inhibit the activity of this enzyme by irreversible modification of the peripheral thiol

FIG. 5. Strain-dependent labeling of the reductase subunit with [³H]tetrahydrocerulenin. Culture portions (0.75 ml) of the *V. harveyi* wild type (lane 1) and mutants M17, M42, A16, and AFM (lanes 2 through 5, respectively), grown to an A_{660} of 1.4 to 1.6, were incubated for 30 min with 0.6 mCi of [³H]tetrahydrocerulenin. Protein samples were prepared and analyzed by SDS-PAGE and fluorography as described in the Fig. 4 legend and in the text. The migration position of the reductase subunit (R [57 kDa]) is shown.
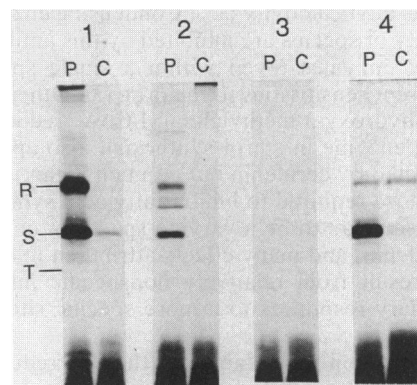
involved in catalytic activity (13). Condensing enzymes from a wide variety of species are inhibited by this antibiotic (19), but different enzymes (even within a single species) can exhibit different sensitivities to the drug (3). Other enzymes, such as 3-hydroxy-3-methylglutaryl-CoA reductase (the rate-limiting enzyme in sterol synthesis), also appear to be directly affected by cerulenin but at much higher concentrations than those required to inhibit fatty acid synthesis (18). Thus, there appears to be a varying spectrum of sensitivity toward cerulenin, and many effects attributed to this antibiotic could result from relatively nonspecific inhibition or from secondary responses to a more specific site of inhibition.

This investigation has established that the reductase subunit responsible for aldehyde synthesis in the luminescent bacterium *V. harveyi* is a primary target of cerulenin under physiological conditions. Thus, our results extend previous studies which indicated that aldehyde synthesis (i.e., fatty acid reduction) is inhibited by the antibiotic (11). A key to the above demonstration is the observation that [^{3}H]tetrahydrocerulenin labeling of the R subunit can be selectively blocked by preincubation with unlabeled cerulenin. Unlike Roberts and Leadlay (21), we have not been able to positively identify any additional proteins (such as β-ketoacyl-ACP synthetase) that are specifically modified with [^{3}H] tetrahydrocerulenin (Fig. 4); this is not too surprising given the high background of labeling in our system and the fact that the reductase enzyme is more abundant than most other enzymes in induced luminescent bacteria (31). In agreement with our conclusions, recent studies have shown that cerulenin reacts directly with the purified R subunit from *Photobacterium phosphoreum* (L. Wall, personal communication).

The observation that luminescence is at least as sensitive to cerulenin as fatty acid synthesis in the M17 mutant raises the possibility that the R enzyme and β-ketoacyl-ACP synthetase share some common mechanistic or structural (or both) features at the active site. Previous studies have indeed indicated that reductase activity involves acylation and deacylation at a site on the enzyme which is sensitive to thiol reagents (32). However, caution must be exercised in the comparison of cerulenin sensitivities in vivo; the present data show that the apparent sensitivity of R to cerulenin may be a function of the level of substrate protection with fatty acid under various conditions. For example, the reduced labeling of the wild-type enzyme with [^{3}H]tetrahydrocerulenin (Fig. 5) can also be obtained with M17 cells preincubated with myristic acid (Fig. 4). Thus, the flux of acyl groups through the active site (and perhaps also the structural integrity of the mutant enzyme complex) must be considered before more specific conclusions can be drawn from an in vivo comparison.

On the basis of the present data, we propose that the aldehyde-stimulable A16 and M42 mutants of *V. harveyi* are blocked at different steps in the fatty acid reduction pathway. The lack of acylation of either the S or R subunit with [^{3}H]myristic acid in the A16 mutant (Fig. 6), coupled with the sensitivity of R to labeling with [^{3}H]tetrahydrocerulenin (Fig. 5), suggests that the defect in this strain involves the activation of fatty acid to acyl-AMP or the subsequent acylation of the synthetase subunit at a Cys residue in the active site (24). On the other hand, the lack of acyl turnover at the R subunit in the M42 mutant (Fig. 6) and the further indication that the acylation site in this enzyme may be blocked to the action of cerulenin in vivo (Fig. 5) are consistent with a defect at this site, perhaps in the reduction
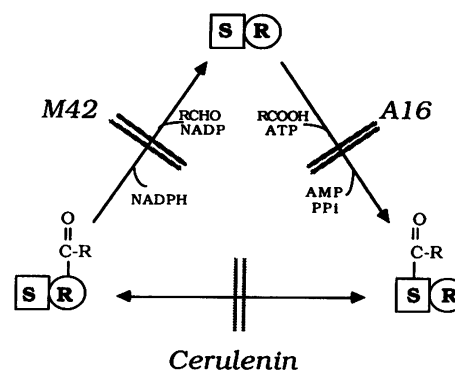

Cerulenin

FIG. 7. Model of the fatty acid reductase pathway showing the proposed sites of cerulenin inhibition and of defects in the *V. harveyi* A16 and M42 mutants.

of acyl-R by NADPH (i.e., in the off-loading of acyl groups from this site). Since cerulenin specifically decreases acylation of the R subunit with [^{3}H]myristic acid (and under certain circumstances increases acylation of the S subunit [Fig. 3]), it would appear to act by preventing the transfer of fatty acyl groups from S to R. The above interpretations are summarized in the model shown in Fig. 7; this model is based on the enzymatic steps elucidated using *P. phosphoreum* (24) and assumes equivalent reactions in *V. harveyi*. Further work is required to test and extend these hypotheses, perhaps by studying the purified enzymes from *P. phosphoreum* or by cloning and sequencing the mutant enzymes from *V. harveyi*, as has been recently achieved for the T subunit from the M17 mutant (17).

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