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Elongation of Exogenous Fatty Acids by the Bioluminescent Bacterium *Vibrio harveyi*

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Bioluminescent bacteria require myristic acid ($C_{14:0}$) to produce the myristaldehyde substrate of the light-emitting luciferase reaction. Since both endogenous and exogenous $C_{14:0}$ can be used for this purpose, the metabolism of exogenous fatty acids by luminescent bacteria has been investigated. Both *Vibrio harveyi* and *Vibrio fischeri* incorporated label from [1-^{14}C]myristic acid ($C_{14:0}$) into phospholipid acyl chains as well as into CO_2. In contrast, *Photobacterium phosphoreum* did not exhibit phospholipid acylation or β-oxidation using exogenous fatty acids. Unlike *Escherichia coli*, the two *Vibrio* species can directly elongate fatty acids such as octanoic ($C_{8:0}$), lauric ($C_{12:0}$), and myristic acid, as demonstrated by radio-gas liquid chromatography. The induction of bioluminescence in late exponential growth had little effect on the ability of *V. harveyi* to elongate fatty acids, but it did increase the amount of $C_{14:0}$ relative to $C_{16:0}$ labeled from [^{14}C]$C_{8:0}$. This was not observed in a dark mutant of *V. harveyi* that is incapable of supplying endogenous $C_{14:0}$ for luminescence. Cerulenin preferentially decreased the labeling of $C_{16:0}$ and of unsaturated fatty acids from all ^{14}C-labeled fatty acid precursors as well as from [^{14}C]acetate, suggesting that common mechanisms may be involved in elongation of fatty acids from endogenous and exogenous sources. Fatty acylation of the luminescence-related synthetase and reductase enzymes responsible for aldehyde synthesis exhibited a chain-length preference for $C_{14:0}$, which also was indicated by reverse-phase thin-layer chromatography of the acyl groups attached to these enzymes. The ability of *V. harveyi* to activate and elongate exogenous fatty acids may be related to an adaptive requirement to metabolize intracellular $C_{14:0}$ generated by the luciferase reaction during luminescence development.

The mechanism and regulation of fatty acid and phospholipid biosynthesis in *Escherichia coli* has been the subject of intense investigation for several years (19, 23). Most studies have indicated that fatty acids synthesized de novo are incorporated into phospholipids via fatty acyl-acyl carrier protein (acyl-ACP) intermediates (24), while coenzyme A (CoA) appears to be the acyl donor for fatty acids derived from exogenous sources (8, 14). Both are effective in vitro substrates for glycerol-3-phosphate acyltransferase, the enzyme catalyzing the first committed step in phospholipid biosynthesis (12). The observation that *E. coli* cannot elongate exogenous fatty acids (26) has been taken as evidence that phospholipid synthesis from endogenous and exogenous acyl precursors proceeds by two distinct pathways, with no appreciable exchange of acyl intermediates (via a free fatty acid pool, for example).

In luminescent marine bacteria such as *Vibrio harveyi*, luciferase catalyzes the light-emitting oxidation of myristaldehyde and reduced flavin mononucleotide to produce flavin mononucleotide and free myristic acid ($C_{14:0}$); for a review, see references 13 and 15. Endogenous $C_{14:0}$ for aldehyde synthesis is normally supplied from acyl-ACP by a luminescence-specific acyltransferase (4, 6); dark mutants defective in this enzyme can also use exogenous $C_{14:0}$ for luminescence (29, 30). The fate of $C_{14:0}$ produced by luciferase is unknown: it could be recycled to form aldehyde by the synthetase (S) and reductase (R) subunits of the fatty acid reductase complex (15, 25), or it could be activated intracellularly and reincorporated into a biosynthetic or degradative pathway of fatty acid metabolism (3a). These pathways would have to be regulated so that intracellular fatty acid or aldehyde does not accumulate during the rapid induction of bioluminescence in late exponential growth (13, 15).

In light of the apparent ability of *V. harveyi* to utilize both endogenous and exogenous $C_{14:0}$ for bioluminescence, the metabolism of exogenous fatty acids by luminescent bacteria has been investigated. The results indicate that in *V. harveyi* and *Vibrio fischeri*, unlike *E. coli*, fatty acids have direct access to both biosynthetic and degradative pathways of fatty acid metabolism.

MATERIALS AND METHODS

Materials. Sodium [1-^{14}C]acetate (58 Ci/mol), octanoic acid ($C_{8:0}$, 54 Ci/mol), and palmitic acid ($C_{16:0}$, 54 Ci/mol) were purchased from Dupont Canada Ltd. (NEN Products, Lachine, Quebec, Canada); [1-^{14}C-labeled lauric ($C_{12:0}$, 59 Ci/mol) and myristic (60 Ci/mol) acids were from Amersham Canada Ltd.. (Oakville, Ontario, Canada). 9,10-^{3}H-labeled myristic and palmitic acids (30 Ci/mmol) were prepared (Amersham) by tritiation of the monounsaturated fatty acid and were purified by thin-layer chromatography (TLC) before use. Cerulenin, boron fluoride-methanol, and molecular weight markers for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Sigma Chemical Co. (St. Louis, Mo.). All unlabeled fatty acid and phospholipid standards were from Sigma or Serdary Research Labs (London, Ontario, Canada). Protosol and En^{3}Hance were from New England Nuclear Corp., and reverse-phase TLC plates (Whatman KC_{18}) were from Mandel Scientific (Rockwood, Ontario, Canada). Silica TLC plates (Redi-Plate G) and all organic solvents (HPLC grade) were obtained from Fisher Scientific (Dartmouth, Nova Scotia, Canada).

Bacterial strains. All strains used in this study, including *V. harveyi* B392 and dark mutants derived by nitrosoguanidine mutagenesis (7), *V. fischeri* ATCC 7744, *Photobacterium phosphoreum* NCMB 844, and *E. coli* RR1, were kindly provided by E. A. Meighen, McGill University (Montreal, Quebec, Canada).

Growth and labeling conditions. *V. harveyi* and *E. coli* were grown at 27°C in a rotary shaker (180 rpm) in complex medium (3a) containing 1% NaCl and 0.2% glycerol as a carbon source. *V. fischeri* and *P. phosphoreum* were grown in complex medium containing 3% NaCl at 27 and 19°C, respectively. Cell growth was monitored by A_{660}, and 1 A_{660} unit corresponds to the number of cells in 1 ml of culture at A_{660} of 1. For *V. harveyi* this was calibrated as 5×10^8 cells. Light emission was monitored using a photomultiplier photometer; 1 light unit (LU) is defined as 5×10^9 quanta s^{-1} (3a). The luminescence of the M17 mutant was measured in the presence of 100 μM $C_{14:0}$.

Typical labeling experiments were performed by adding a sample of labeled fatty acid stock solution in ethanol (<1% final volume) to a 1-ml culture sample in a glass tube. Incubation was carried out under the growth conditions described above.

Lipid extraction and separation. In most experiments, incubation was stopped by addition of 3.75 volumes of methanol-chloroform (2:1, vol/vol), and lipids were extracted by the method of Bligh and Dyer (2) as outlined previously (3a). When removal of labeled fatty acid substrate was desired, cells were centrifuged (15,000 × *g*, 3 min) and washed with growth medium containing 2% bovine serum albumin prior to lipid extraction.

TLC separation of individual phospholipid components was achieved on silica G TLC plates (20 by 20 cm) by development in chloroform-methanol-acetic acid-water (85:15:10:3.5, vol/vol). In routine analyses of labeled fatty acids associated with total phospholipid, or for quantitation of free fatty acid or aldehyde, plates were developed in petroleum ether-diethyl ether-acetic acid (70:30:1, vol/vol). Radioactive material remaining at the origin in this solvent system was essentially all phospholipid and was scraped and used to prepare samples for fatty acid analysis as outlined below. The distribution of radioactivity on TLC plates was determined by using a Bioscan System 200 imaging detector; peaks were identified by position relative to lipid standards. The integration of each peak (as a percentage of total) and the amount of lipid extract applied were used to calculate the radioactivity associated with individual components (3a).

Fatty acid analysis. Fatty acid methyl esters were prepared by reaction with 10% BF_3 in methanol and separated by radio-gas-liquid chromatography (radio-GLC) as outlined previously (3a), using a Perkin-Elmer Sigma 300 gas chromatograph equipped with a Nuclear-Chicago 5190 radioactivity flow monitor. Fatty acids were identified from the retention times of ^{14}C-fatty acid methyl ester standards or of unlabeled standards monitored simultaneously by a flame ionization detector. The integrated output of the radioactivity detector and the disintegrations per minute extracted with petroleum ether after transmethylation were used to quantitate ^{14}C-fatty acids formed. The initial precursor-specific radioactivity was used to calculate level of incorporation, and no molar correction for the expected number of labeled acetate units per fatty acid chain using different precursors has been applied.

Measurement of CO_2 formation. Culture samples (2 ml) were incubated with radiolabeled fatty acid in 25-ml Erlenmeyer flasks equipped with center wells (Kontes) and rubber stoppers (14). Incubation was stopped on ice, and Protosol (0.2 ml) was injected into the center well, followed by 0.2 ml of 1 M citric acid added to the culture (final pH, <4). After further incubation for 30 min at 37°C, $^{14}CO_2$ trapped in the center well was determined by liquid scintillation counting. Blank values (labeled fatty acid added to growth medium alone) were subtracted from sample values to determine $^{14}CO_2$ production. The citric acid-acidified culture remaining was used for lipid extraction and determination of fatty acid label incorporation into phospholipids; results obtained in this manner were indistinguishable from those obtained using normal incubation procedures (described above).

SDS-PAGE analysis. Culture samples (1 ml) were incubated for 10 min with radiolabeled fatty acid and then centrifuged (15,000 × *g*, 5 min), and cell pellets were stored at −20°C. Preparation of in vivo fatty acid-labeled samples for SDS-PAGE was carried out exactly as described by Wall et al. (30), except that 2-mercaptoethanol was omitted from the SDS sample buffer. SDS-PAGE and fluorography were performed as described previously (30).

To analyze ^3H-labeled fatty acids attached to the S or R subunits of fatty acid reductase, the corresponding gel slices (detected by fluorography) were hydrated in H_2O, crushed with a glass rod, and incubated with 1 ml of 0.1 M KOH in methanol for 30 min at 37°C in the presence of 5 μg each of unlabeled $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, and $C_{18:0}$. Samples were acidified with HCl and extracted with petroleum ether. This initial extract was further treated with BF_3 (as described above) to ensure complete methylation. ^3H-labeled fatty acid methyl esters were separated on reverse-phase TLC plates by development in acetic acid-acetonitrile (1:1, vol/vol) and visualized by fluorography after spraying with En^{3}Hance. Fatty acids were identified by comigration with ^{14}C-labeled fatty acid methyl ester standards prepared under identical conditions.

RESULTS

Metabolism of exogenous myristic acid by luminescent bacteria. Most investigations of bacterial luminescence have involved *V. harveyi*, *V. fischeri*, or *P. phosphoreum* (15). To study the metabolism of exogenous fatty acids in these gram-negative bacteria, cultures of all three species (and *E. coli* for comparison) were incubated with [1-^{14}C]$C_{14:0}$ in complex medium containing glycerol; incorporation of radioactivity into total phospholipids and CO_2 was monitored (Table 1). The results indicated that both *Vibrio* species (like *E. coli*) can utilize exogenous long-chain fatty acids for phospholipid acylation as well as for β-oxidation. In contrast, no appreciable accumulation of myristate label in phospholipid or CO_2 was observed for *P. phosphoreum*, suggesting that this species lacks the enzymes necessary for the uptake, activation, or utilization of extracellular fatty acids. Incorporation of myristic acid into phospholipid was greatest with *V. harveyi*, the subject of previous studies on fatty acid metabolism and bioluminescence (3a), and thus this species was chosen for further investigation.

Although *V. harveyi* is known to grow on agar with acetate as the sole carbon and energy source (18), growth of batch cultures in minimal medium containing $C_{14:0}$ (1 mM) or sodium acetate (0.4%) was exceedingly slow: after a growth lag of several days, the A_{660} doubled every 2 to 3 days (data not shown). In contrast, *V. harveyi* will grow quite well in minimal medium containing 0.2% glycerol as a carbon source (5), doubling every 2 to 3 h. Incorporation of fatty acid label into *V. harveyi* in complex growth medium was

TABLE 1. Incorporation of radioactivity from [1-^{14}C]myristic acid into phospholipids and CO_2 by various bacterial species[a]

Bacterial species	[^{14}C]$C_{14:0}$ incorporation (nmol/A_{660} unit) into:	
	Phospholipid	CO_2
V. harveyi (5)	14.2 ± 1.5	3.0 ± 0.7
V. fischeri (6)	4.1 ± 1.0	3.1 ± 0.3
P. phosphoreum (4)	0.03 ± 0.01	0[b]
E. coli (4)	11.3 ± 0.9	7.0 ± 0.3

[a] Samples (2 ml) of each species were incubated for 30 min with [1-^{14}C]$C_{14:0}$ (0.24 μCi) at a final concentration of 50 μM, and the incorporation of radioactivity into total phospholipid and CO_2 was determined as outlined in the text. The data are the mean and standard deviation of *n* samples, where *n* is shown in parentheses. Data for each strain were compiled from two or three separate experiments: *V. harveyi* (A_{660} = 1.6 to 2.0), *V. fischeri* (A_{660} = 0.70 to 1.0), *P. phosphoreum* (A_{660} = 1.3 to 2.0), and *E. coli* (A_{660} = 0.6 to 0.7).
[b] Sample radioactivity was less than or equal to that of blank values.

linear with time and was unaffected by preincubation (1 h) of cells with unlabeled $C_{14:0}$ (data not shown), indicating that fatty acid uptake was not inducible over the time periods used in this investigation. Although details of the regulation of fatty acid metabolism remain to be studied, it seems that aspects of this metabolism are different in *V. harveyi* from those in *E. coli* (19, 23).

Direct elongation of exogenous fatty acids by *V. harveyi*. The acyl chain products of 1-^{14}C-fatty acid incorporation into total *V. harveyi* phospholipids were determined by radio-GLC. [1-^{14}C]$C_{14:0}$ was substantially (25 to 35%) elongated to $C_{16:0}$, while [1-^{14}C]$C_{12:0}$ was extensively elongated to $C_{14:0}$ and $C_{16:0}$, with no desaturation to $C_{16:1}$ and $C_{18:1}$ (Fig. 1). Elongation of [1-^{14}C]$C_{16:0}$ was not observed ($C_{16:0}$ is the longest saturated fatty acid found in *V. harveyi* [3a]). Moreover, less than 2% of phospholipid-associated label arising from [U-^{14}C]$C_{16:0}$ appeared as $C_{14:0}$ (data not shown), indicating a minimal amount of chain shortening prior to transacylation. Elongation of [^{14}C]$C_{14:0}$ to $C_{16:0}$ was also observed in *V. fischeri*, while the observation that *E. coli* cannot directly elongate exogenous fatty acids (26) was repeated under the present conditions (data not shown).

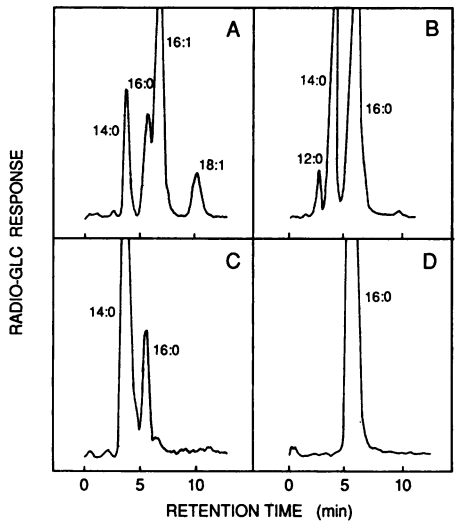

FIG. 1. Radio-GLC analysis of labeled *V. harveyi* acyl chains derived from exogenous 1-^{14}C-fatty acids. Luminescence-induced wild-type cells (A_{660} = 2.0; 500 LU/ml) were incubated for 10 min (1 μCi in 1 ml; 16 to 18 μM final concentration) with 1-^{14}C-labeled $C_{8:0}$ (A), $C_{12:0}$ (B), $C_{14:0}$ (C), or $C_{16:0}$ (D). The total phospholipid fraction from TLC was used to prepare fatty acid methyl esters as described in the text. The temperature program for radio-GLC was 186°C for 8 min, followed by an increase of 5°C/min to 210°C, and then constant at this temperature. Attenuation of the integrator was adjusted for each sample to obtain the tracings shown.

TABLE 2. Chain length composition and phospholipid distribution of *V. harveyi* fatty acid label originating from [1-^{14}C]$C_{14:0}$[a]

Phospholipid[b]	^{14}C-fatty acid (pmol/10^6 cells)	
	$C_{14:0}$	$C_{16:0}$
Phosphatidylethanolamine (90%)	3.30 ± 0.28	1.70 ± 0.54
Phosphatidylglycerol (8%)	0.23 ± 0.06	0.21 ± 0.09
Cardiolipin (2%)	0.09 ± 0.02	0.03 ± 0.01

[a] Culture samples (2 ml) of induced *V. harveyi* (A_{660} = 2.4; 750 LU/ml) were incubated with 16 μM [1-^{14}C]$C_{14:0}$ (2 μCi) for 10 min. Phospholipids were isolated by TLC and individually transmethylated. Fatty acid methyl esters (90 to 98% of recovered radioactivity) were analyzed by radio-GLC; $C_{14:0}$ and $C_{16:0}$ accounted for >98% of the total integrated profile. The mean and standard deviation of four samples are shown.
[b] The percentage of total phospholipid-associated fatty acid label is included in parentheses for each component.

Incubation of *V. harveyi* with [1-^{14}C]$C_{8:0}$ resulted in the formation of phospholipid containing all the major saturated and unsaturated fatty acids occurring in this species (Fig. 1). The possibility of [1-^{14}C]$C_{8:0}$ degradation followed by de novo resynthesis from [^{14}C]acetate was excluded as incubation in the presence of 25 mM unlabeled sodium acetate produced only a modest (<10%) decrease in the incorporation of [^{14}C]$C_{8:0}$ into fatty acids with no change in the product label distribution. These results probably indicate that *V. harveyi* uses the same mechanism of unsaturated fatty acid synthesis as *E. coli*: introduction of a double bond at the 10-carbon stage by 3-hydroxydecanoyl-ACP dehydrase (23).

TLC analysis revealed that the majority (>95%) of the cell-associated lipid label originating from exogenous ^{14}C-fatty acid was associated with the phospholipid fraction; negligible labeled free fatty acid or aldehyde was detected when *V. harveyi* cells were washed in medium containing bovine serum albumin before lipid extraction. Radioactivity from [1-^{14}C]$C_{14:0}$ was associated with each of the three major phospholipid components, phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin (Table 2), and upon transesterification it was recovered primarily as fatty acid methyl esters. Moreover, labeled $C_{16:0}$ elongated from [^{14}C]$C_{14:0}$ was found in all phospholipid species. Further analysis of acyl chain turnover and positional distribution will be required to determine whether additional phospholipid-specific pathways for exogenous fatty acids, such as the deacylation/reacylation of *E. coli* phosphatidylethanolamine (22), are present in *V. harveyi*.

Effects of bioluminescence development on fatty acid elongation. The ability of *V. harveyi* to elongate exogenous fatty acid did not appear to be specifically related to the induction of bioluminescence enzymes in late exponential growth. Elongation of [1-^{14}C]$C_{8:0}$ to form saturated and unsaturated fatty acids occurred in wild-type cells labeled both before and after induction of luminescence (Fig. 2), although the overall rate was less for induced cells in transition from exponential to stationary phase. Similar results were obtained with [1-^{14}C]$C_{14:0}$ (data not shown). Moreover, fatty

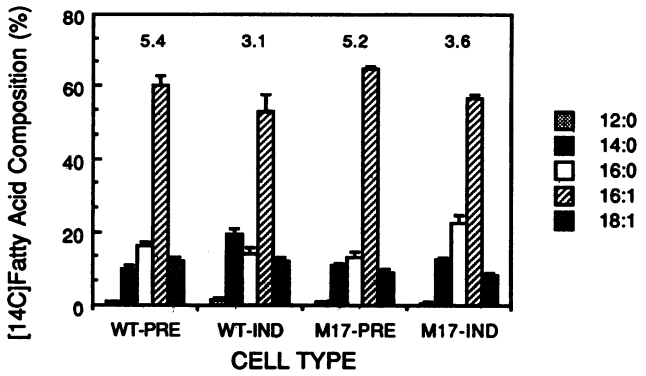

FIG. 2. The fatty acid products of [1-^{14}C]$C_{8:0}$ incorporation into *V. harveyi* wild-type (WT) and mutant M17 phospholipids. Samples (1 ml) from preinduced (PRE) (A_{660} = 0.4; 2 to 3 LU/ml) and induced (IND) (A_{660} = 1.8; 500 to 600 LU/ml) cultures were incubated with 18 μM [1-^{14}C]$C_{8:0}$ (1 μCi) for 10 min. Total phospholipid isolated by TLC was used to prepare samples for radio-GLC analysis. The incorporation of labeled precursor into total fatty acids (picomoles per 10^6 cells) is shown for each cell type. The mean and standard deviation of four determinations are shown.

acid elongation was observed in *V. harveyi* dark mutants that are defective in the supply of endogenous $C_{14:0}$ (mutant M17; Fig 2), in the reduction of $C_{14:0}$ to myristaldehyde (mutant A16; 30), or in all luminescence functions (mutant AFM; 6).

On the other hand, the induction of bioluminescence did appear to affect the elongation products of shorter-chain fatty acids such as [^{14}C]$C_{8:0}$. Induced wild-type cells exhibited a significant increase in the ratio of $C_{14:0}/C_{16:0}$ labeled with this precursor (Fig. 2). This was not observed in the M17 mutant, which lacks the acyl-ACP acyltransferase (T) required to couple bioluminescence and de novo fatty acid synthesis (6). Growth-related changes in the percent labeling of other fatty acids (notably a decrease in $C_{16:1}$) were also observed, but to a similar extent in both wild-type and mutant strains (Fig. 2).

Effects of cerulenin on fatty acid elongation. The effects of cerulenin, a known inhibitor of β-ketoacyl-ACP synthase (3), were examined to study further the nature of exogenous fatty acid elongation by *V. harveyi* (Table 3). When luminescent cells were preincubated with cerulenin at concentrations that do not inhibit the growth of *V. harveyi* (28), the incorporation of labeled acyl groups into phospholipid was decreased with [^{14}C]$C_{8:0}$ as a precursor, but was unchanged or even increased with the longer-chain ^{14}C-fatty acids. Radio-GLC analysis of the products indicated an almost total inhibition of $C_{16:0}$ formation from all precursors, as well as decreased unsaturated fatty acid synthesis from [^{14}C]$C_{8:0}$. Parallel experiments with [^{14}C]acetate revealed a pattern of inhibition generally similar to that observed for [^{14}C]$C_{8:0}$, although a more detailed comparison of the levels and products of incorporation suggests a certain degree of complexity in the inhibition by cerulenin of reactions involving these precursors. This could result from the differential involvement of more than one condensing enzyme, each with differing sensitivity to cerulenin (3). Nevertheless, these results indicate that cerulenin has comparable effects both on the elongation of exogenous fatty acids and on de novo fatty acid synthesis.

Fatty acylation of *V. harveyi* proteins: chain length specificity. Previous studies have demonstrated that the synthetase (S, 42 kilodaltons [kDa]) and reductase (R, 57 kDa) enzymes involved in aldehyde synthesis for luminescence in

TABLE 3. Effects of cerulenin on the incorporation of ^{14}C-lipid precursors into *V. harveyi* phospholipids: analysis of the labeled acyl products formed[a]

^{14}C-labeled precursor	Cerulenin	Label incorporation (pmol/10^9 cells) into:				
		$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:1}$
$C_{14:0}$	−		1,260	490	ND[b]	ND
	+		2,420	24	ND	ND
$C_{12:0}$	−	50	530	860	ND	ND
	+	210	790	30	ND	ND
$C_{8:0}$	−	ND	610	520	1,980	380
	+	26	120	16	19	ND
Acetate	−	ND	23	32	123	35
	+	11	95	ND	24	ND

[a] Wild-type cells were grown to A_{660} = 1.5 (230 LU/ml); at this time the culture was split and further incubated in the presence of 10 μg of cerulenin per ml (+) or an equivalent volume (10 μl) of ethanol (−). After 1 h, duplicate 1-ml samples from each flask were labeled for 10 min with 1 μCi (16 to 18 μM) of ^{14}C-fatty acid or 10 μCi (170 μM) of [^{14}C]acetate. Fatty acid methyl esters were prepared from the total phospholipid fraction and analyzed by radio-GLC. The average of duplicate analyses is shown.

[b] ND, Not detected or trace (<1% of total fatty acid label).

V. harveyi can be labeled with [^{3}H]$C_{14:0}$ in vivo (30). The fatty acid specificity of enzyme acylation was analyzed by incubating cells with various ^{14}C-labeled fatty acids, followed by SDS-PAGE and fluorography to visualize labeled proteins (Fig. 3). The most intense labeling of the S and R acyl enzyme intermediates was obtained with myristic acid, reflecting the specificity of bioluminescence for this fatty acid (11, 15, 29). Labeling of additional proteins was also noted, particularly with [^{14}C]$C_{8:0}$ and [^{14}C]$C_{12:0}$, which labeled proteins in the 48-to-52 and 15-to-20 kDa ranges. *V. harveyi* acyl-ACP migrates in the latter molecular size range (6), but no positive identification of the proteins labeled by $C_{8:0}$ and $C_{12:0}$ has been made.

To evaluate the extent of interconversion of fatty acids that participate in the luminescence reactions, the labeled fatty acids involved in S and R subunit acylation from exogenous [^{3}H]$C_{14:0}$ and [^{3}H]$C_{16:0}$ were analyzed by reverse-phase TLC and fluorography (Fig. 4). Virtually no chain length conversion of [^{3}H]$C_{14:0}$ prior to enzyme acylation was observed for either the S or R subunit, whereas about one-third of the label derived from [^{3}H]$C_{16:0}$ appeared as the shortened product $C_{14:0}$. This experiment emphasizes the different chain length specificities of enzyme versus phospholipid acylation in *V. harveyi*, since the opposite (i.e., elongation of $C_{14:0}$ but not shortening of $C_{16:0}$) was observed in the latter process (see Fig. 1).

DISCUSSION

Bacteria lack the degree of intracellular compartmentalization found in eucaryotic cells and must therefore employ other methods of maintaining separate regulation of biosynthetic and degradative metabolic pathways. In *E. coli* fatty acid metabolism, this is achieved by using chemically distinct acyl intermediates: acyl-ACP for de novo synthesis and acyl-CoA for degradation of exogenous fatty acids. A 43-kDa transport protein and acyl-CoA synthetase, products of the *fadL* (1) and *fadD* (14) genes, respectively, are responsible for exogenous fatty acid uptake and activation to form acyl-CoA that can serve as a substrate for phospholipid acylation or β-oxidation. Acyl-CoA thioesterases (27) and

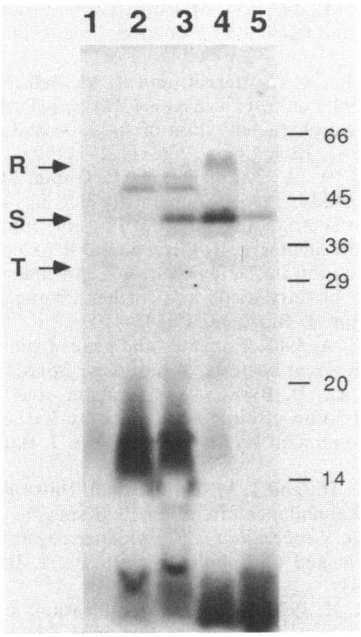

FIG. 3. Fatty acylation of *V. harveyi* proteins in vivo with different 1-^{14}C-labeled fatty acid precursors. Samples (1 ml) of *V. harveyi* culture (A_{660} = 2.1; 600 LU/ml) were incubated for 10 min with 1-^{14}C-labeled sodium acetate (lane 1), $C_{8:0}$ (lane 2), $C_{12:0}$ (lane 3), $C_{14:0}$ (lane 4), or $C_{16:0}$ (lane 5). Concentrations used were 32 to 36 μM (2 μCi/ml) except for [^{14}C]acetate (345 μM, 20 μCi/ml). Proteins were separated by SDS-PAGE and visualized by fluorography (2 months) as described in the text; each lane contained 130 μg of protein. Migration of protein standards (in kilodaltons) and of the fatty acid reductase R, S, and T subunits is indicated on the right- and left-hand sides, respectively.

acyl-ACP synthetase (21), enzymes which could in theory combine to deliver exogenous fatty acids to biosynthetic pools, do not appear to be involved in *E. coli* phospholipid synthesis from these fatty acids (8).

The present investigation has established that the pathways of exogenous fatty acid metabolism in the luminescent bacterium *V. harveyi* are different from those in *E. coli*. Unlike the latter species, commonly used for studies of bacterial fatty acid metabolism, *V. harveyi* (and *V. fischeri*) can utilize exogenous fatty acid in both degradative (β-oxidation) and biosynthetic (elongation) pathways. These species also appear to differ in the regulation of exogenous fatty acid uptake. The present results are interesting in light of the otherwise similar lipid metabolism of *E. coli* and *V. harveyi*. Both organisms are gram-negative, facultative anaerobes with comparable fatty acid and phospholipid compositions (3a, 9, 23). Acyl-ACP from either species is a substrate for the T acyltransferases from both *V. harveyi* and *P. phosphoreum* (6). Moreover, the luminescence operons from *V. fischeri* (10) and *V. harveyi* (16) can be expressed in *E. coli*, although good expression of the latter operon is only observed in the presence of an unknown host mutation (16). The present results indicate that the ability of *V. harveyi* to elongate exogenous fatty acids is not specifically related to the induction of bioluminescence, although this activity could be responsible for elongation of intracellular free myristic acid generated by the luciferase reaction.

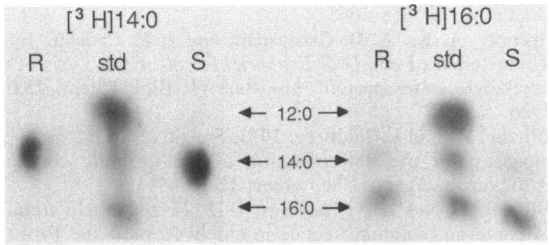

FIG. 4. Identification of enzyme-bound ^{3}H-fatty acids from *V. harveyi* cells labeled in vivo with [^{3}H]$C_{14:0}$ and [^{3}H]$C_{16:0}$. Culture samples (1 ml) of induced *V. harveyi* (see Fig. 3) were incubated for 10 min with 1.2 μM (36 μCi) of ^{3}H-fatty acid, and proteins were separated by SDS-PAGE. Fatty acid methyl esters were prepared from gel slices containing the labeled R and S enzymes. Samples containing 4,000 to 12,000 dpm were fractionated by reverse-phase TLC and visualized by fluorography (47 days) as outlined in the text. The center lanes contained methyl esters of ^{14}C-labeled $C_{12:0}$, $C_{14:0}$, and $C_{16:0}$ (10,000 dpm total).

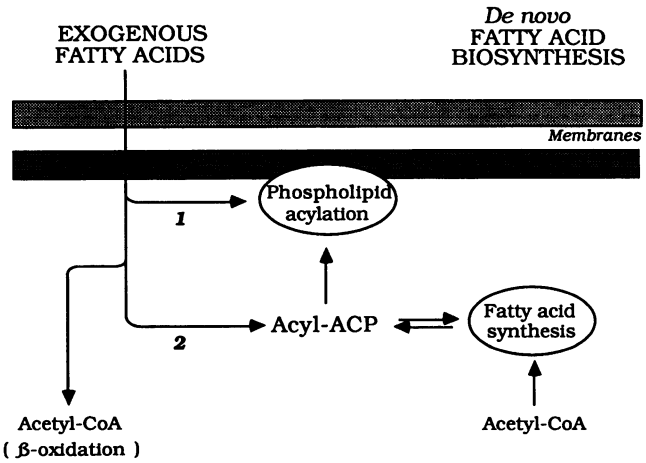

FIG. 5. Proposed model of fatty acid metabolism in *V. harveyi*. The present data support a model in which exogenous fatty acids can be incorporated into *V. harveyi* phospholipids by direct transacylation (1) or after incorporation into biosynthetic pools (2). Only the former route is accessible in *E. coli*. The nature of the intracellular acyl intermediates in *V. harveyi*, including those which interact with the luminescence system, has not been established.

The observation that *P. phosphoreum* does not utilize exogenous fatty acids for phospholipid acylation or β-oxidation is not surprising since this fastidious species can grow only on a limited number of carbon sources which does not include acetate (18). Nevertheless, it has been demonstrated that *P. phosphoreum* S and R enzymes can be labeled in vivo with [^{3}H]$C_{14:0}$ (15, 30). Thus, it appears either that fatty acid transport in this organism does not involve activation to an acyl intermediate (i.e., acyl-CoA) or that such an intermediate is precluded from participation in general lipid metabolic pathways, but not bioluminescence.

A general model of fatty acid metabolism in *V. harveyi* and its relationship to that in *E. coli* is proposed in Fig. 5. Several lines of evidence suggest that exogenous fatty acids can be activated intracellularly as acyl-ACP thioesters in *V. harveyi* and are elongated by enzymes similar to, or possibly identical with, those involved in de novo biosynthesis. First, the profiles of labeled fatty acids formed from endogenous (acetate) and exogenous ($C_{8:0}$) precursors are quite similar, although not identical (see Table 3; also compare Fig. 2 and Table 2 from reference 3a). Second, the effects of cerulenin on the elongation of different fatty acid precursors are consistent with a common set of enzymes acting on acyl

intermediates derived from intracellular and extracellular sources. Moreover, the preferential inhibition of unsaturated fatty acid synthesis and of elongation of $C_{14:0}$ to $C_{16:0}$ by cerulenin is similar to the results reported previously for de novo synthesis in *E. coli* (3). Finally, exogenous fatty acids which are extensively elongated (such as $C_{8:0}$ and $C_{12:0}$) appear to produce a distinct set of labeled acyl proteins, some of which may in fact be acyl-ACP thioesters. Although the involvement of ACP in exogenous fatty acid metabolism must still be regarded as tentative, *V. harveyi* extracts do possess acyl-ACP synthetase activity (6). *E. coli* acyl-ACP synthetase has recently been implicated in a special reacylation pathway for lysophosphatidylethanolamine (22; pathway not shown in Fig. 5), but it may play a more expanded role in activating intracellular free fatty acids (17, 20). Further work is clearly required to elucidate details of *V. harveyi* fatty acid metabolism and to determine how this bacterium can regulate the fate of intracellular acyl intermediates in biosynthetic and degradative pathways.

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