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Purification and Characterization of Fatty Acyl-Acyl Carrier Protein Synthetase from *Vibrio harveyi*

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A *Vibrio harveyi* enzyme which catalyzes the ATP-dependent ligation of fatty acids to acyl carrier protein (ACP) has been purified 6,000-fold to apparent homogeneity by anion-exchange, gel filtration, and ACP-Sepharose affinity chromatography. Purified acyl-ACP synthetase migrated as a single 62-kDa band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and as an 80-kDa protein by gel filtration under reducing conditions. Activity of the purified enzyme was lost within hours in the absence of glycerol and low concentrations of Triton X-100. Acyl-ACP synthetase exhibited K_ms for myristic acid, ACP, and ATP of 7 μM, 18 μM, and 0.3 mM, respectively. The enzyme was specific for adenine-containing nucleotides, and AMP was the product of the reaction. No covalent acyl-enzyme intermediate was observed. Enzyme activity was stimulated up to 50% by iodoacetamide but inhibited >80% by *N*-ethylmaleimide: inhibition by the latter was prevented by ATP and ACP but not myristic acid. Dithiothreitol and sulfhydryl-directed reagents also influenced enzyme size, activity, and elution pattern on anion-exchange resins. The function of acyl-ACP synthetase has not been established, but it may be related to the capacity of *V. harveyi* to elongate exogenous fatty acids by an ACP-dependent mechanism.

Most metabolic processes involving fatty acids are preceded by activation of the fatty acid to form a thioester derivative: the cellular location and product of the activation process usually define the metabolic fate of the fatty acid. At least three distinct mechanisms of fatty acid activation occur in *Escherichia coli* (reviewed in reference 16). Endogenous fatty acids are synthesized as derivatives of acyl carrier protein (ACP), attached to the phosphopantetheine moiety of this small (9-kDa) dissociable protein. Depending upon their acyl chain length, acyl-ACP intermediates undergo further elongation or serve as acyl donors in the synthesis of phospholipids (11), lipopolysaccharides (1), or acyl proteins (14). In contrast, exogenous fatty acids are transported across the outer membrane by a specific protein (2, 3) and activated to form acyl coenzyme A (acyl-CoA) by acyl-CoA synthetase at the inner membrane (17, 21). Acyl-CoA intermediates are targeted towards β-oxidation and phospholipid acylation (9, 11). Limited activation of exogenous fatty acids to acyl-ACP also occurs during phosphatidylethanolamine turnover catalyzed by *E. coli* 2-acyl-glycerophosphoethanolamine acyltransferase–acyl-ACP synthetase (23), but these intermediates do not dissociate from the enzyme in vivo, nor do they exchange with the biosynthetic acyl-ACP pool (8). A fourth mechanism of activation exists in marine bioluminescent bacteria, in which ATP-dependent activation of myristic acid (14:0) and autoacylation by the synthetase subunit of fatty acid reductase channel this fatty acid to synthesis of the myristyl aldehyde substrate of the light-emitting luciferase reaction (reviewed in reference 20). Myristic acid for this process is normally diverted from fatty acid synthesis by a specific myristoyl-ACP esterase (7), although it can also be obtained exogenously in mutants defective in this enzyme (31, 32).

We have obtained evidence for an additional pathway of fatty acid activation in the luminescent bacterium *Vibrio harveyi*. This organism is capable of using exogenous fatty acids for both β-oxidation and phospholipid acylation, but (unlike in *E. coli* [28]) these fatty acids can also be elongated in a cerulenin-sensitive biosynthetic pathway (5). We have recently demonstrated that intracellular acyl-ACP intermediates are labeled with exogenous [^{3}H]14:0; this labeling does not arise from complete degradation of the fatty acid, as [^{3}H]acetate produces a different labeled acyl-ACP profile (27a). These observations have led to the discovery of an enzyme activity in extracts of *V. harveyi* which catalyzes the ATP-dependent ligation of fatty acids to ACP (6, 27). Although the role of this enzyme has not been established, its soluble nature and broad fatty acid chain length specificity (C_6 to C_{16}) indicate that it is not homologous to *E. coli* acyl-ACP synthetase.

In the present study, *V. harveyi* acyl-ACP synthetase has been purified to homogeneity and partially characterized. The enzymatic and structural features of this 62-kDa enzyme suggest that it may play a novel role in activation of free fatty acids within the cell.

MATERIALS AND METHODS

Materials. [9,10-^{3}H]myristic acid (25 Ci/mmol) was obtained by thin-layer chromatographic purification of the products of tritiation of myristoleic acid prepared by Amersham Canada Ltd. (Oakville, Ontario, Canada). [α-^{32}P]ATP (800 Ci/mol) and En^{3}Hance were purchased from Du Pont-NEN (Du Pont Canada Inc., Mississauga, Ontario, Canada). Myristic acid, 2-mercaptoethanol, ATP, dATP, GTP, ITP, CTP, UTP, 5′-adenylyl-imidodiphosphate, *E. coli* ACP (used without further purification), and native molecular weight standards for gel filtration were obtained from Sigma Chemical Co. (St. Louis, Mo.). Dithiothreitol (DTT) was obtained from Boehringer-Mannheim Canada Ltd. (Laval, Quebec, Canada). Triton X-100 (Surfact-Amps) and reduced Triton X-100 were obtained, respectively, from Aldrich Chemical Co. (St. Louis, Mo.) and from Pierce Chemical

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Co. (Rockford, Ill.). A Bio-Gel HPHT column and all equipment for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were from Bio-Rad (Canada) Ltd. (Mississauga, Ontario, Canada). CNBr-Sepharose, DEAE-Sepharose Fast Flow, Sephacryl S-300 HR, and molecular weight standards for SDS-PAGE were obtained from Pharmacia (Canada) Ltd. (Montreal, Quebec, Canada). ACP-Sepharose was prepared by using 5 mg of *E. coli* ACP and 0.5 g of CNBr-Sepharose according to the manufacturers' instructions. Protein-Pak DEAE-5PW and 300SW columns for the Waters 650 protein chromatograph were from Waters (Canada) Ltd. (Mississauga, Ontario, Canada). All other chemicals were reagent grade or better.

Purification of acyl-ACP synthetase. The M17 mutant of *V. harveyi* B392, which lacks myristoyl-ACP esterase activity (7), was used as a source of enzyme. Cells were grown in a rotary shaker at 27°C in complex medium containing (per liter) 10 g of NaCl, 5 g of Bacto-Tryptone, 0.5 g of yeast extract, 2 ml of glycerol, 3.7 g of Na_2HPO_4, 1 g of KH_2PO_4, 0.5 g of $(NH_4)_2HPO_4$, and 0.2 g of $MgSO_4 \cdot 7H_2O$. Bacteria were harvested in late log phase as described previously (27), and cell pellets were stored at −20°C and used within 1 month.

All preparative procedures were carried out at 4°C unless otherwise stated. Cell paste from 5 liters of culture was thawed and resuspended in 50 ml of buffer A (20 mM Tris-HCl, pH 7.5, 10% glycerol) and disrupted by sonication (eight 30-s bursts), followed by centrifugation at 7,000 × *g* for 30 min (27). The resulting pellet was resuspended in 25 ml of buffer A, resonicated (four 45-s bursts), and centrifuged as described above. The combined supernatants were applied to a column containing DEAE-Sepharose Fast Flow equilibrated with buffer A (Fig. 1A). The column was washed with buffer A followed by a linear gradient of 0 to 0.5 M NaCl in buffer A. Fractions containing acyl-ACP synthetase activity were pooled and made 75% saturated with solid ammonium sulfate. The resulting precipitate was dissolved in 2 ml of buffer A containing 1 mM EDTA, clarified by centrifugation (15,000 × *g*, 10 min), and applied to a Sephacryl S-300 HR column (95 by 1.5 cm) equilibrated in the same buffer; the flow rate was 10 ml/h, and 2-ml fractions were collected. Acyl-ACP synthetase eluted with an apparent mass of 300 to 500 kDa and was stable for several months at this stage (27).

For further purification, the Sephacryl S-300 HR pool was applied to a Protein-Pak DEAE-5PW column at room temperature through the pump of a Waters 650 protein chromatograph. The column was eluted with a multistep linear NaCl gradient in buffer A (Fig. 1B). Two peaks of acyl-ACP synthetase activity (I and II) were always observed. Fractions containing the larger peak (II) of activity were pooled, and reduced Triton X-100 was added to a final concentration of 0.002% (wt/vol). The sample was centrifugally concentrated to approximately 1 ml with a Centricon-30 filter (Amicon) and applied directly to an ACP-Sepharose column equilibrated with 0.1 M NaH_2PO_4 (pH 7.0)–10% glycerol–0.002% reduced Triton X-100 (buffer B) at room temperature (Fig. 2). The flow was stopped for 10 min after the sample entered the column and then was resumed at a rate of 5 ml/h. The column was eluted with 5 ml of buffer B followed by 5 ml of 0.5 M NaH_2PO_4 (pH 7.0)–10% glycerol–0.002% reduced Triton X-100. Fractions containing acyl-ACP synthetase activity were pooled, concentrated to <0.5 ml with a Centricon-30 filter, and stored at −20°C after addition of 0.1 mM DTT.

Enzyme assays. *V. harveyi* acyl-ACP synthetase activity was monitored by conversion of [^{3}H]14:0 to [^{3}H]14:0-ACP in a filter disk assay (22). Fractions containing enzyme were incubated at 37°C with *E. coli* ACP (20 μM), 0.1 M Tris-HCl (pH 7.8), 10 mM $MgSO_4$, 10 mM ATP, 5 mM DTT, and 80 μM [^{3}H]myristic acid (1 Ci/mmol) in a total volume of 25 μl. After 20 min, 20 μl was removed and applied to Whatman 3MM filter paper disks (2 cm^2) which were washed three times with methanol-chloroform-acetic acid (6:3:1, vol/vol) to remove unbound fatty acid. The [^{3}H]myristoyl-ACP product was counted in a liquid scintillation spectrometer with an efficiency of 16% (27). Luciferase activity was measured as the peak light emission obtained following injection of $FMNH_2$ into a solution containing decyl aldehyde and the fraction as outlined previously (12).

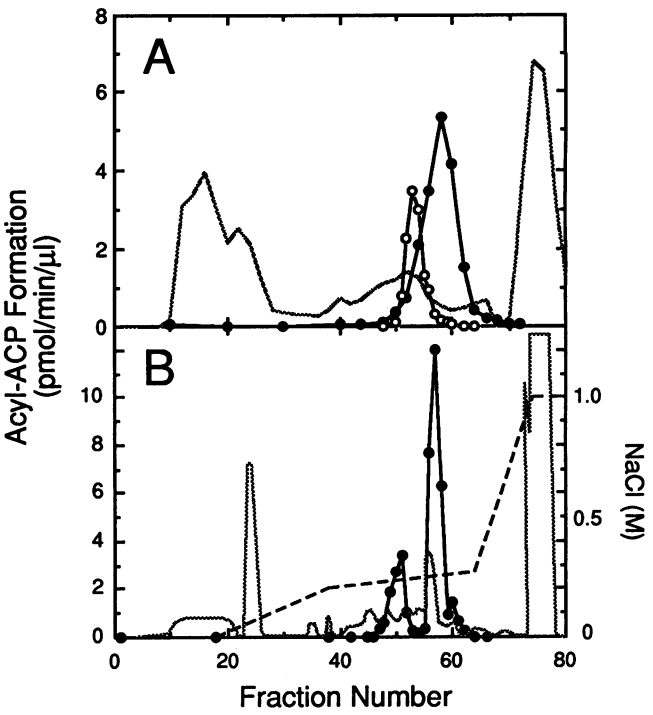

FIG. 1. DEAE anion-exchange chromatography of *V. harveyi* acyl-ACP synthetase. (A) Initial fractionation of mutant M17 cell extract on a DEAE-Sepharose column (16 by 2.5 cm). The sample (750 mg of protein in 60 ml of buffer A) was applied to the column, washed with 100 ml of buffer A, and then eluted with a linear gradient (700 ml total) of 0 to 0.5 M NaCl in buffer A, starting at fraction 17. The flow rate was 1 ml/min, and 10-ml fractions were collected. (B) Separation of activity into two peaks on Protein-Pak DEAE-5PW. Sephacryl S-300 HR pool (2.4 mg of protein in 12 ml) was applied to the column (7.5 by 0.75 cm) in buffer A plus 50 μM DTT and eluted with a multistep gradient to 1 M NaCl (– – –) in this buffer. The flow rate was 1 ml/min, and 1-ml fractions were collected. Activities of acyl-ACP synthetase (●) and luciferase (○) (in light units per microliter, where 1 light unit equals 5 × 10^9 quanta per s) were measured, as was A_{280} (shaded line; full range, 20 and 0.2 U for panels A and B, respectively).

Miscellaneous methods. Samples were prepared for SDS-PAGE (18) by boiling for 3 min in SDS sample buffer (27) containing 2 mM DTT, followed by addition of *N*-ethylmaleimide to a final concentration of 10 mM. Proteins were separated on 10% polyacrylamide gels and detected by using a Bio-Rad silver staining kit. In some cases, proteins were precipitated from very dilute solution with 5 volumes of acetone prior to sample preparation. Protein levels were quantitated by the micro-bicinchoninic assay (Pierce).

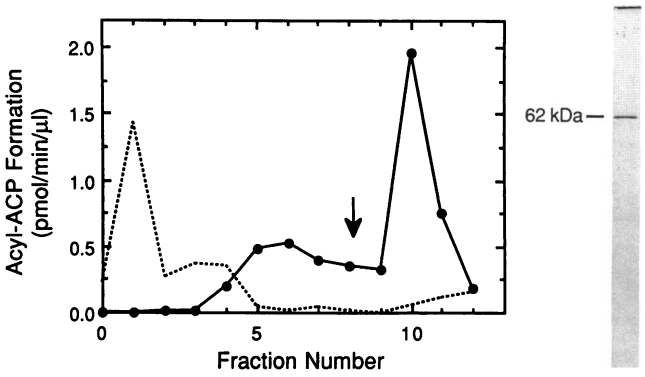

FIG. 2. Isolation of acyl-ACP synthetase activity by affinity chromatography on *E. coli* ACP-Sepharose. Active fractions from DEAE-5PW peak II were concentrated to 1 ml and applied to an ACP-Sepharose column (2 by 1 cm) in 0.1 M NaH_2PO_4 (pH 7.0)–10% glycerol–0.002% reduced Triton X-100 as described in the text. The column was eluted with buffer containing 0.5 M NaH_2PO_4 (see arrow). The flow rate was 5 ml/h, and 1-ml fractions were collected. Acyl-ACP synthetase activity (●) and A_{280} (10^1 U) (– – –) are shown. On the right is SDS-PAGE analysis of fraction 10 after concentration by Centricon-30.

RESULTS

Purification of *V. harveyi* acyl-ACP synthetase. In a previous study (27), we noted that *V. harveyi* acyl-ACP synthetase was quite stable after partial purification by Cibacron Blue and gel filtration chromatography but exhibited a substantial loss of activity upon further purification. An improved purification scheme in which DEAE-Sepharose was used instead of Cibacron Blue as the initial purification step was devised. Acyl-ACP synthetase activity was found in a single broad peak which eluted after luciferase in a NaCl gradient (Fig. 1A). After ammonium sulfate precipitation and gel filtration on Sephacryl S-300 HR as described previously (27), the enzyme was applied to a high-performance anion-exchange column (Fig. 1B). Invariably, two peaks of activity (I and II) were observed with a shallow NaCl gradient. The larger second peak (peak II) exhibited much higher specific activity and was used for further purification. The key final step in the preparation was affinity chromatography on *E. coli* ACP-Sepharose (Fig. 2). Most acyl-ACP synthetase activity was eluted in 0.5 M phosphate buffer with very little or no contamination with other proteins. Some breakthrough of activity on this column was apparently due to weak binding of the enzyme to the matrix, as this activity could be concentrated and reapplied to the column with complete binding. As shown in Table 1, an increase in specific activity of 6,000-fold was achieved in the four chromatographic steps with a yield of 1 to 2% of starting activity.

Purified acyl-ACP synthetase exhibited a single band on SDS-PAGE under reducing conditions (Fig. 2); the apparent mass of this protein was 62 ± 1 kDa (average ± standard deviation from four preparations). To verify that activity was associated with this component, enzyme obtained from ACP-Sepharose was further chromatographed by either high-performance gel filtration (Protein-Pak 300SW) or hydroxylapatite (Bio-Gel HPTP) chromatography. In both cases, a single peak of activity which, after acetone precipitation and SDS-PAGE of fractions, correlated with a 62-kDa band was observed (data not shown). Although no activity could be recovered from gel slices following SDS-PAGE (8), acyl-ACP synthetase activity did comigrate with the major silver-stained band on native polyacrylamide gels.

The low yield of acyl-ACP synthetase can be at least partially attributed to the extreme lability of the purified enzyme. During our initial attempts at purification using ACP-Sepharose, we observed that activity was completely lost within hours unless trace amounts of Triton X-100 were added to the enzyme preparation. Concentrations of Triton X-100 as low as 0.001% (10-fold lower than the critical micelle concentration) prevented enzyme inactivation, with maximal stabilization (≥100% recovery of activity relative to the original column fraction) observed with 0.01 to 0.1% detergent. Enzyme activity was inhibited if Triton X-100 was >0.1% in the final assay mixture (6). As a compromise chosen to stabilize activity yet avoid interference with the assay and column performance, reduced Triton X-100 (0.002%) was routinely added to all buffers for the final ACP-Sepharose step. In the presence of this detergent and 10% glycerol, which is required at all preparative stages (27), acyl-ACP synthetase exhibited an about 50% loss of activity in 1 month at −20°C. The presence of DTT (0.1 mM) improved stability about twofold when added at this stage but was found to have a negative effect on overall yield when included at earlier preparative steps.

Enzymatic properties of acyl-ACP synthetase. The K_ms of affinity-purified acyl-ACP synthetase for myristic acid (7 μM) and *E. coli* ACP (20 μM) were similar to that measured for the crude enzyme (6). The K_m for *V. harveyi* ACP was identical to that for *E. coli* ACP. Enzyme activity was half-maximal at 0.3 mM ATP, with inhibition observed at ATP concentrations greater than 10 mM, also in agreement with earlier data (6). However, while those studies indicated some activity of the crude enzyme with GTP, purified acyl-ACP synthetase was very specific for adenine-containing nucleotides: dATP exhibited 25% of the activity of ATP, while much lower activity was observed for GTP (0.6%) with no detectable activity for ITP, CTP, or UTP (all concentrations at 10 mM Mg^{2+}-nucleotide). Although most long-chain fatty acid ligases form AMP as the nucleotide product (including *E. coli* acyl-ACP synthetase [22]), 5′-adenylyl-imidodiphosphate was only 2% as effective as ATP as a substrate for the *V. harveyi* enzyme. Nevertheless, thin-layer chromatographic analysis of the reaction mixture using [α-^{32}P]ATP as the substrate revealed that AMP is indeed the nucleotide product (Fig. 3). No covalent acyl-enzyme intermediate was detected when acyl-ACP synthetase was incu-

TABLE 1. Purification of *V. harveyi* acyl-ACP synthetase

Purification step	Total protein (mg)	Total activity (nmol/min)	Sp act (nmol/min/mg)	Purification (fold)	Yield (%)
Cell extract	1,440	1,100	0.76	1	100
DEAE-Sepharose	61	450	7.4	9.7	41
Sephacryl S-300 HR	37	330	8.9	12	30
DEAE-5PW	1.0	116	116	152	10
ACP-Sepharose	0.003	13.7	4,600	6,000	1.2

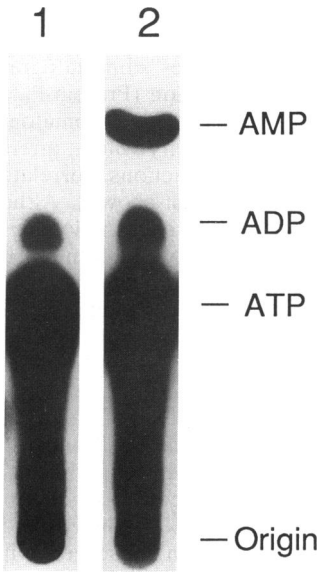

FIG. 3. Nucleotide product of the *V. harveyi* acyl-ACP synthetase reaction. [α-^{32}P]ATP (178 Ci/mol, 100 μM final concentration) was incubated with 44 μM *E. coli* ACP and 80 μM unlabeled myristic acid in the absence (lane 1) or presence (lane 2) of affinity-purified acyl-ACP synthetase for 20 min as described in the text. Ten microliters of reaction mixture was spotted on a Silica Gel G plate and developed in isobutyric acid-water-ammonia (66:33:1, vol/vol). Labeled nucleotide product was visualized by autoradiography. Migration of ATP, ADP, and AMP standards is indicated; only the bottom half of the chromatograph is shown.

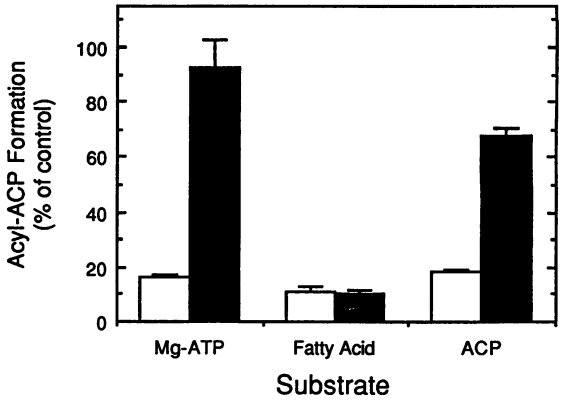

FIG. 4. Substrate protection of acyl-ACP synthetase activity against inactivation by *N*-ethylmaleimide. Sephacryl S-300-purified enzyme (20 μl) was incubated at room temperature with substrate alone (control), with *N*-ethylmaleimide (90 mM final concentration), or with both substrate and *N*-ethylmaleimide. The substrate concentration in the last incubation was 20 mM Mg-ATP, 100 μM 14:0, or 40 μM ACP. At 30 min, substrate or inhibitor was added as necessary to achieve identical composition for each set (final volume, 24 to 28 μl). Duplicate 2-μl aliquots were immediately removed and mixed with acyl-ACP synthetase assay mixture (22 μl, containing 10 mM DTT). The enzyme assay was initiated with [^{3}H]14:0, and activities from samples treated with *N*-ethylmaleimide in the absence (white bars) and presence (solid bars) of substrate were expressed as percentages of the control activity (1.8 to 2.6 pmol/min/μl). The means and ranges from duplicate assays are shown.

bated with [^{3}H]14:0 (±ATP or ACP) and analyzed by SDS-PAGE and fluorography.

Affinity-purified acyl-ACP synthetase was inhibited 80% in the presence of 1% Triton X-100 or 0.5 M LiCl in the assay, as expected from the behavior of the enzyme in cell extracts (6). A number of other potential modulators were tested to further elucidate the nature of the acyl-ACP synthetase reaction and the role of specific amino acid groups in catalysis. No effect on activity was observed in the presence of cerulenin (50 μg/ml), phenylmethylsulfonyl fluoride (10 mM), CoA (50 μM), and malonyl-, acetyl-, or palmitoyl-CoA (20 μM). Interestingly, activity of the purified enzyme was stimulated up to 50% by preincubation (30 min) with 20 mM iodoacetamide, while *N*-ethylmaleimide (another sulfhydryl-specific reagent) produced 80% inhibition at this concentration. In an experiment using partially purified (Sephacryl S-300 HR) enzyme, preincubation with either ACP or ATP afforded protection against *N*-ethylmaleimide inactivation while myristic acid had no effect (Fig. 4). The greater effect of ATP than ACP in this experiment was attributed to the presence of a much higher concentration of this substrate relative to its apparent K_m (see above).

Properties of acyl-ACP synthetase: role of sulfhydryl groups. The conflicting effects of DTT on enzyme yield versus stability and of sulfhydryl inhibitors on activity prompted us to further examine the influence of sulfhydryl groups on the physical properties of acyl-ACP synthetase. Although DTT did not appreciably alter the elution of the enzyme as monitored at the Sephacryl S-300 HR gel filtration step, it had a pronounced effect on elution of the enzyme on Protein-Pak 300SW following ACP-Sepharose chromatography. In the absence of DTT, most acyl-ACP synthetase activity eluted in a peak corresponding to 330 kDa (average from two experiments), with a trailing shoulder at a lower molecular mass (Fig. 5). In contrast, adding DTT to the column buffer resulted in a pronounced decrease in the activity of the higher-molecular-mass peak, leaving the peak of lower activity (80 kDa). The amount of protein in these

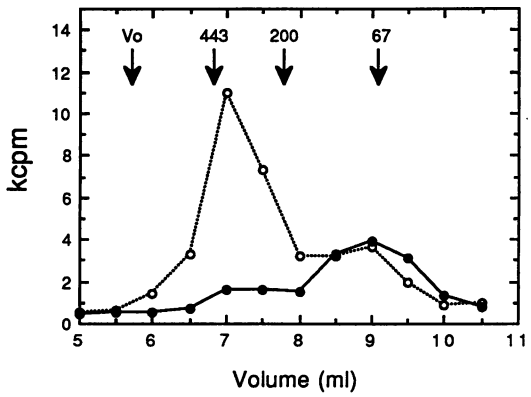

FIG. 5. Effect of DTT on elution of acyl-ACP synthetase activity by gel filtration. Affinity-purified enzyme was injected (0.1-ml loop) onto a Protein-Pak 300SW column (30 by 0.8 cm) equilibrated with 0.1 M Na_2PO_4 (pH 7.0)–10% glycerol–0.01% reduced Triton X-100 in the absence (○) or presence (●) of 10 mM DTT. The flow rate was 0.5 ml/min, and 0.5-ml fractions were collected for measurement of acyl-ACP synthetase activity (shown as kilocounts per minute from a 5-μl fraction added to the standard assay). The peak elutions of standards blue dextran (Vo), apoferritin (443 kDa), β-amylase (200 kDa), and bovine serum albumin (67 kDa) are shown; these were not appreciably affected by DTT. The results are representative of two separate experiments.

experiments was too small to detect a corresponding change in the elution of enzyme by measuring the A_{280}.

Agents that modify sulfhydryl groups also affected the elution of acyl-ACP synthetase activity on DEAE-5PW columns (Fig. 1B). We observed that 10 mM DTT increased activity associated with peak I by 1.5- to 2-fold, while treatment of the starting sample (Sephacryl S-300 HR pool) with 10 mM iodoacetamide decreased or removed this peak entirely (data not shown). Moreover, dialysis and rechromatography of either peak I or peak II on DEAE-5PW resulted in the reappearance of both peaks. Although attempts to isolate and identify the enzyme associated with peak I were not successful because of its low activity, these observations suggest that the same 62-kDa protein may be responsible for both peak I and peak II activities. This was further indicated by the similar substrate requirements and inhibition of both DEAE peak I and peak II activities by Triton X-100 and LiCl in the assay.

DISCUSSION

The present study reports the isolation of a novel fatty acid-activating enzyme: *V. harveyi* acyl-ACP synthetase. A key to the purification of this labile 62-kDa enzyme is the use of ACP affinity chromatography. Although binding of the enzyme to ACP is relatively weak (likely a reflection of the K_m of 20 μM for this substrate) and quantitative binding to the column was not always observed, this step resulted in a 40-fold purification. The other key to isolation of the enzyme is the use of Triton X-100 to stabilize it at late stages of preparation. We believe that this effect results from interaction of detergent monomers with a hydrophobic site on the surface of the enzyme, possibly preventing it from aggregation or nonspecific binding to surfaces at dilute concentrations. Whether this site is equivalent to the fatty acid binding site has not been determined.

Sulfhydryl groups on acyl-ACP synthetase also appear to have an influence on its catalytic properties, structure, and stability. In particular, we have obtained evidence for a sulfhydryl group which is essential for enzyme activity and can be blocked by *N*-ethylmaleimide. While we cannot rule out the possibility that another type of functional group is involved (because of the high concentrations of *N*-ethylmaleimide required and the lack of inhibition by iodoacetamide), the effects of ATP and ACP in blocking this inhibition could also be indicative of a sulfhydryl group which is buried in a substrate binding pocket and is thus poorly reactive. The role of this group in the mechanism is unknown, and it does not appear to be an acceptor for the acyl group following activation. This differs from the acyl-protein synthetase involved in aldehyde synthesis in *Photobacterium phosphoreum*, in which fatty acid activation via an acyl-AMP intermediate (25) is followed by autoacylation of the enzyme at a cysteine residue at a separate site (26, 30).

Another class of sulfhydryl groups also appears to be involved in the structure of acyl-ACP synthetase. Treatment of partially purified enzyme with DTT or iodoacetamide affects its elution on anion-exchange columns. Moreover, DTT causes the loss of an aggregated (or, less likely, a very asymmetric) but more active form of the affinity-purified enzyme, leaving a less active and apparently monomeric form. Whether this results from reduction of intermolecular disulfide bonds or from decreased noncovalent interactions in response to reduction of intramolecular bonds is not known. However, these observations might explain the negative effect of reducing agents on the yield of enzyme activity. They also raise the possibility that, despite the presence of a single 62-kDa band after purification, acyl-ACP synthetase is part of a complex involving other proteins in vivo. ACP is one obvious candidate in this regard: we have found that acyl-ACP synthetase activity can be specifically precipitated from *V. harveyi* cell extracts by using anti-*V. harveyi* ACP polyclonal antibody; up to 10% of the initial activity in the cell extract is recovered when the immunoprecipitates are washed with the same buffer (0.5 M phosphate) used to elute the enzyme from ACP-Sepharose (27b). The effects of sulfhydryl reagents on acyl-ACP synthetase activity and structure could also reflect mechanisms involved in regulation of fatty acid activation by an enzyme complex. It is interesting that multisubunit allosteric enzymes such as malate dehydrogenase (29) and aspartate transcarbamylase (10) exhibit activation in response to reagents such as iodoacetamide.

The only other enzyme that has been reported to catalyze the ATP-dependent ligation of fatty acids to ACP is *E. coli* 2-acyl-glycerophosphoethanolamine acyltransferase–acyl-ACP synthetase (8, 22). This 27-kDa integral inner membrane enzyme catalyzes both the activation and transfer of exogenous fatty acids to lyso-phosphatidylethanolamine (8, 13) and is involved in reacylation of the 1-position of phosphatidylethanolamine during synthesis of outer membrane lipoproteins (15, 23, 24). Acyl-ACP is a tightly bound intermediate of the *E. coli* enzyme and is released only at unphysiological concentrations of LiCl and other salts, allowing the synthetase partial reaction to be monitored (8). On the basis of its soluble nature, different fatty acid chain length specificity (6, 27), and response to LiCl (6), *V. harveyi* acyl-ACP synthetase appears to play a role different from that of the *E. coli* enzyme in fatty acid metabolism. The present study also shows that the *V. harveyi* enzyme is substantially larger and exhibits a much higher catalytic rate than *E. coli* acyl-ACP synthetase (8). The absence of any effects of CoA derivatives and cerulenin indicates that *V. harveyi* acyl-ACP synthetase activity is not due to acyl-CoA synthetase or to fatty acid biosynthetic enzymes that catalyze acyl-ACP formation via acyl-CoA transacylation (19).

The isolation of *V. harveyi* acyl-ACP synthetase will facilitate the development of probes to test the function of the enzyme in vivo. By analogy to other fatty acid-activating enzymes, such as *E. coli* acyl-ACP synthetase, acyl-CoA synthetase, and the acyl-protein synthetase of luminescent bacteria, it is likely that the *V. harveyi* enzyme channels acyl groups to specific metabolic pathways in this organism. We have previously suggested that an acyl-ACP synthetase activity could be involved in reactivation of intracellular 14:0 produced by luciferase oxidation of myristyl aldehyde, since induction of bioluminescence does not result in accumulation of this fatty acid or major changes in the acyl composition of phospholipids (4). However, acyl-ACP synthetase is constitutive and not coinduced with luminescence enzymes (6), appears to be absent in other luminescent bacteria such as *P. phosphoreum* (6), and exhibits a much broader chain length preference than would be expected for such a function. On the other hand, relative activities of the enzyme in *V. harveyi* and *Vibrio fischeri* (6) do appear to correlate with the capacities of these organisms to elongate exogenous fatty acids by an ACP-dependent mechanism (5). The physiological function of this metabolic process, which is not observed in *E. coli* (28), and any role of acyl-ACP synthetase remain to be established.

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