JOURNAL OF BACTERIOLOGY, Jan. 1996, p. 571–573 Vol. 178, No. 2
0021-9193/96/$04.00+0

Isolation of *Vibrio harveyi* Acyl Carrier Protein and the *fabG*, *acpP*, and *fabF* Genes Involved in Fatty Acid Biosynthesis

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Received 30 August 1995/Accepted 6 November 1995

We report the isolation of *Vibrio harveyi* acyl carrier protein (ACP) and cloning of a 3,973-bp region containing the *fabG* (encoding 3-ketoacyl-ACP reductase, 25.5 kDa), *acpP* (encoding ACP, 8.7 kDa), *fabF* (encoding 3-ketoacyl-ACP synthase II, 43.1 kDa), and *pabC* (encoding aminodeoxychorismate lyase, 29.9 kDa) genes. Predicted amino acid sequences were, respectively, 78, 86, 76, and 35% identical to those of the corresponding *Escherichia coli* proteins. Five of the 11 sequence differences between *V. harveyi* and *E. coli* ACP were nonconservative amino acid differences concentrated in a loop region between helices I and II.

Acyl carrier protein (ACP) is a highly conserved fatty acid carrier essential for the synthesis of fatty acids, phospholipids, and other complex molecules in a variety of organisms. The prototypic ACP from *Escherichia coli* is a 9-kDa acidic protein (pI, 4.1) of 77 amino acids which carries fatty acids as thioester intermediates attached to a phosphopantetheine prosthetic group at Ser-36 (17, 31). In addition to its major function as a carrier for activated acyl intermediates during fatty acid elongation, ACP serves as an acyl donor for the synthesis of phospholipids (25), lipid A (1), and protein toxins such as hemolysin (12). ACP or ACP-like proteins have also been implicated in the synthesis of polyketides (27), periplasmic glucans involved in osmotic adaptation in *E. coli* (26), cell-host signaling (NodF factors) in *Rhizobium leguminosarum* (9), and cell wall synthesis in gram-positive bacteria (11). Thus, in a typical bacterial cell as many as a dozen different enzymes, with overlapping acyl chain specificities, compete for a small pool of acyl-ACP. Consequently, variations in the structure of ACP could have a large influence on the metabolic fate of acyl groups.

In bioluminescent bacteria such as *Vibrio harveyi*, ACP is also involved in supplying myristic acid for the synthesis of the myristaldehyde substrate for luciferase (4, 6). A soluble fatty acyl-ACP synthetase which may be responsible for reentry of myristic acid into fatty acid biosynthetic pathways and/or activation of exogenous fatty acids to acyl-ACP in *V. harveyi* (5, 28) has been isolated (29). The present investigation was initiated to determine whether additional or different roles of ACP in a bioluminescent bacterium might be associated with particular structural features of the protein.

Purification of *V. harveyi* ACP. ACP was purified from wild-type *V. harveyi* B392 grown in complex medium containing 1% NaCl (28) by using a modification of the isopropanol extraction procedure and batch chromatography on DEAE-Sepharose described for *E. coli* ACP (24). Further purification included chromatography on Sephacryl S-200 and Waters DEAE-5PW columns. ACP concentration was monitored by conversion to ^{3}H-acyl-ACP with partially purified *V. harveyi* acyl-ACP synthetase (29). Final purification was 1,700-fold, with a typical yield of 60 mg of ACP per kilogram (wet weight) of *V. harveyi* cells. Purified *V. harveyi* ACP migrated as a single 20-kDa band on a sodium dodecyl sulfate (SDS)-polyacrylamide gel, similar to *E. coli* ACP (M_r, 8,847), which is known to exhibit anomalous migration in this gel system (13).

The amino acid sequence of the N-terminal 55 residues of purified *V. harveyi* ACP blotted to an Immobilon membrane was determined by Edman degradation on an Applied Biosystems 473A Sequencer (Department of Biochemistry, Queen's University, Kingston, Ontario, Canada). Comparison with the corresponding *E. coli* ACP sequence (21) over this region revealed considerable similarity between the two proteins, except for one region between amino acid residues 18 and 25, where five residues were different between the two species.

Isolation of the *V. harveyi acpP* gene region. To clone the *V. harveyi acpP* gene, we designed a degenerate 26-mer oligonucleotide based on the region of limited sequence identity (residues 18 to 26) noted above; this oligonucleotide did not hybridize with *Pst*I-digested *E. coli* genomic DNA or MR24 plasmid DNA (containing *E. coli acpP* [21]). A recombinant clone (pUSS11) containing a 1.4-kb *Eco*RI-*Eco*RI insert in pUC18 was obtained from a size-selected *V. harveyi* library. Sequencing revealed that pUSS11 contained only two-thirds of the *V. harveyi acpP* gene from the 5′ end (Fig. 1); the deduced amino acid sequence was identical to that obtained by protein sequencing, except that the N-terminal methionine was missing in the latter. The 3′ end of the *V. harveyi* ACP gene was isolated with a second probe (5′ AAGCTTCTTTCGTTGACG 3′), chosen from the *V. harveyi* ACP nucleotide sequence downstream of a predicted *Hin*dIII site in the *V. harveyi acpP* gene, to enable us to detect an overlapping fragment in a *Hin*dIII-digested *V. harveyi* library. A number of positively hybridizing recombinant clones were selected for DNA sequencing, and one of them, containing a 2.7-kb insert including the 3′ end of the *V. harveyi acpP* gene, was designated pUO1 (Fig. 1).

Complete sequencing of both pUSS11 and pUO1 inserts (total, 3,973 bp) revealed several open reading frames (Fig. 1). Comparison of predicted amino acid sequences with those of corresponding *E. coli* proteins indicated a *V. harveyi* gene organization identical to that in the fatty acid biosynthetic gene cluster of *E. coli* (21). Included in this region were *fabD* (nucleotides 1 to 264), encoding part of a protein 62% identical to the C terminus of *E. coli* malonyl coenzyme A:ACP transacylase

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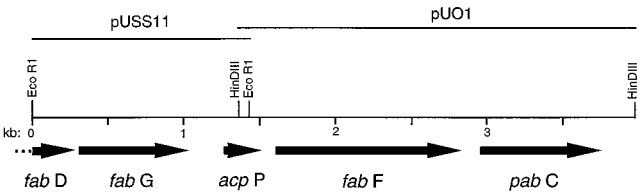

FIG. 1. Map of the *V. harveyi* fatty acid biosynthetic region encoded by overlapping pUSS11 and pUO1 inserts. The relative gene positions and direction of transcription of *fabD*, *fabG*, *acpP*, *fabF*, and *pabC* are indicated (solid arrows). The *Eco*RI sites flanking the pUSS11 insert (positions 1 and 1429) and the *Hin*dIII sites flanking the pUO1 insert (positions 1358 and 3968) are also shown.

(21); *fabG* (nucleotides 302 to 1033), encoding a 25.5-kDa protein 78% identical to *E. coli* 3-ketoacyl-ACP reductase (21); *acpP* (nucleotides 1282 to 1512; see below); and *fabF* (nucleotides 1609 to 2853), encoding a 43.1-kDa protein 76% identical to *E. coli* 3-ketoacyl-ACP synthase II. The latter protein was originally reported as 3-ketoacyl-ACP synthase IV, product of the *fabJ* gene (30), but more recently it has been shown to be identical to *fabF* (18). Given the instability of clones containing *E. coli fabF*, it would be interesting to determine whether the *V. harveyi* gene can complement *E. coli fabF* mutants and confer temperature regulation of *cis*-vaccenic acid synthesis (17). Downstream of *fabF* is *pabC* (nucleotides 2964 to 3776), encoding a 29.9-kDa protein 35% identical to *E. coli* aminodeoxychorismate lyase (10). This enzyme appears to be less conserved between the two species than those involved in fatty acid synthesis.

We observed that the presence of the upstream and 5′ regions of the *acpP* gene in recombinant plasmids such as pUSS11 resulted in a low copy number and poor recovery of plasmid, while pUO1 was expressed at a normal copy number (data not shown). Moreover, attempts to express recombinant *V. harveyi* ACP after restriction and ligation of pUSS11 and pUO1 in the overlap region were unsuccessful, likely because of deleterious effects of ACP expression in host *E. coli*. This has been observed previously for the cloned *E. coli acpP* gene and appears to be due to the inhibition of *sn*-glycerol-3-phosphate acyltransferase upon accumulation of apo-ACP (15).

***V. harveyi* ACP gene and protein sequence.** The nucleotide sequence of *V. harveyi acpP* is 79% identical to that of *E. coli*. Mature *V. harveyi* ACP contains 76 amino acids (molecular mass, 8,734 Da including phosphopantetheine) and has a calculated isoelectric point of pH 3.99. Thus, it is one residue shorter and slightly more acidic than *E. coli* ACP, containing two more acidic residues. Fig. 2 shows alignment of the *V. harveyi* ACP amino acid sequence with other bacterial and

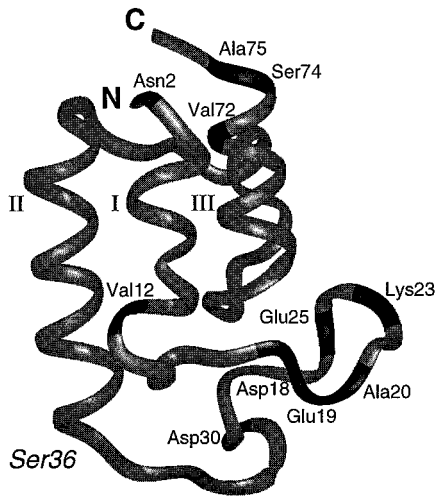

FIG. 3. Tertiary structure of *E. coli* ACP showing amino acid replacements in *V. harveyi*. Coordinates of the *E. coli* ACP B state derived from nuclear magnetic resonance data (16) were obtained from the Protein Data Bank (3) at the Brookhaven National Laboratory, and the tertiary structure was displayed with the Insight II program on an Iris Indigo XS-24 workstation. The amino acid residues which differ between the *V. harveyi* and *E. coli* ACPs (Fig. 2) are shown in black and labeled. The N and C termini, the three major α-helices (I to III), and the phosphopantetheine binding site (Ser-36) are also indicated.

algal ACP sequences. *V. harveyi* ACP has the greatest identity with *E. coli* ACP (86% identical), while others are clearly less similar, consistent with the fact that *V. harveyi* and *E. coli* are the most closely related to each other among these species (33). To examine the predicted locations of amino acid replacements in *V. harveyi* ACP, the tertiary structure of one of the two *E. coli* ACP dynamic states (B state) derived from nuclear magnetic resonance data (16) is shown in Fig. 3. In this model, *E. coli* ACP contains three major α-helices at residues 3 to 14 (I), 37 to 51 (II), and 65 to 75 (III). Helices I and II are linked by a long loop region, part of which (residues 26 to 34) appears to be quite structured on the basis of amide exchange rates (2). Five of the eleven residues which are different between the two ACPs (residues 18 to 20, 23, and 25) are nonconservative replacements found within a short span preceding this structured loop region, all involving changes in charged residues. Both ACPs have identical sequences between residues 31 and 71, including the phosphopantetheine prosthetic group attachment site (Ser-36) and residues thought to be involved in fatty acid binding (14, 19, 23). None of the amino acid differences would be expected to disrupt the major helical

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                                  10        20        30     *  40        50        60        70
Vibrio harveyi                    SNIEERVKKIIVEQLGVD-EAEVKNEASFVDDLGADSLDTVELVMALEEEFDTEIPDEEAEKITTVQAAIDYVNSAQ
Escherichia coli                  ·T·········G·····K-QE··T·N····E·····*·································I·GH·A
Rhizobium meliloti                ·D·A·····VIDH····-AEK·SEG···I·····*·········F····GV····DA·DS·L··GD·VKFIEK··A
Rhodobacter sphaeroides           ·D·AD·····V··H···E-·EK·TETT··I·····*·········F····GI····DA··T·Q·FGD·P
Anabaena variabilis             SQ·ETF·K····VI···S·ENPDT·TP····AN··Q··*···············I·····A··········V·?I·
Cryptomonas                     NEQE·F·K·QT··S·····-KSQ·TKD·N·AN·····*·········I··A·NI····DA··Q·SNL·Q·V·FISQKVAA
Saccharopolyspora erythraea MDRKE·F··IEQVLA····IP-AEQITE··DLRE···M·*··L····S···D·VGMRVEQSQL·G·E··GHVMELTLDL...
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FIG. 2. Sequence comparison of *V. harveyi* ACP and other ACPs. The amino acid sequence of *V. harveyi* ACP is aligned with ACP sequences of *E. coli* (21), *Rhizobium meliloti* (20), *Rhodobacter sphaeroides* (20), *Anabaena variabilis* (8), *Cryptomonas* spp. (32) and *Saccharopolyspora erythraea* (22). Residues identical to *V. harveyi* ACP residues are indicated by dots. The conserved serine (position 36), to which the phosphopantetheine group is attached, is highlighted with a star.

regions, suggesting that the *E. coli* ACP tertiary structure is a reasonable approximation for *V. harveyi* ACP.

The similar structures of *V. harveyi* and *E. coli* ACP would predict that they can substitute for each other in many functions. Indeed, previous work in our laboratory has shown that both ACPs are comparable substrates (K_m, 20 μM) for *V. harveyi* acyl-ACP synthetase (7). Moreover, both *V. harveyi* and *E. coli* acyl-ACPs can be cleaved by *V. harveyi* myristoyl-ACP thioesterase in vitro (6). On the other hand, *V. harveyi* ACP exhibits a lower K_m than *E. coli* ACP in the activation of *E. coli* glucosyltransferase involved in the synthesis of membrane-derived oligosaccharides (30a). *V. harveyi* and *E. coli* ACPs also appear to exhibit differences in mobility on native and SDS gels upon acylation with long-chain fatty acids (5a). The localization of amino acid sequence differences at relatively discrete regions on the surface of *E. coli* and *V. harveyi* ACPs provides an opportunity to define regions of ACP responsible for interaction with specific enzymes.

Nucleotide sequence accession number. The nucleotide sequence reported in this work has been assigned GenBank accession number Vh U39441.

We thank Debra Fice for excellent technical assistance and Frederick Palmer, Harold Cook, and Neale Ridgway for helpful discussions. We are also grateful to Paul Liu, Sook Shin, Christina Lyons, and Tina Inalsingh for protein and DNA sequencing, to Jonathan Parrish for ACP modeling, to Audrey Weissborn for providing unpublished results, and to David Keating for pMR24.

This work was supported by grants from the Medical Research and Natural Sciences and Engineering Research Councils of Canada.

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