Site-directed Mutagenesis of Acyl Carrier Protein (ACP) Reveals Amino Acid Residues Involved in ACP Structure and Acyl-ACP Synthetase Activity*

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Acyl carrier protein (ACP) interacts with many different enzymes during the synthesis of fatty acids, phospholipids, and other specialized products in bacteria. To examine the structural and functional roles of amino acids previously implicated in interactions between the ACP polypeptide and fatty acids attached to the phosphopantetheine prosthetic group, recombinant *Vibrio harveyi* ACP and mutant derivatives of conserved residues Phe-50, Ile-54, Ala-59, and Tyr-71 were prepared from glutathione S-transferase fusion proteins. Circular dichroism revealed that, unlike *Escherichia coli* ACP, *V. harveyi*-derived ACPs are unfolded at neutral pH in the absence of divalent cations; all except F50A and I54A recovered native conformation upon addition of MgCl₂. Mutant I54A was not processed to the holo form by ACP synthase. Some mutations significantly decreased catalytic efficiency of ACP fatty acylation by *V. harveyi* acyl-ACP synthetase relative to recombinant ACP, e.g. F50A (4%), I54L (20%), and I54V (31%), whereas others (V12G, Y71A, and A59G) had less effect. By contrast, all myristoylated ACPs examined were effective substrates for the luminescence-specific *V. harveyi* myristoyl-ACP thioesterase. Conformationally sensitive gel electrophoresis at pH 9 indicated that fatty acid attachment stabilized mutant ACPs in a chain length-dependent manner, although stabilization was decreased for mutants F50A and A59G. Our results indicate that (i) residues Ile-54 and Phe-50 are important in maintaining native ACP conformation, (ii) residue Ala-59 may be directly involved in stabilization of ACP structure by acyl chain binding, and (iii) acyl-ACP synthetase requires native ACP conformation and involves interaction with fatty acid binding pocket residues, whereas myristoyl-ACP thioesterase is insensitive to acyl donor structure.

Although it is best known for its central role as a carrier of acyl intermediates during fatty acid synthesis (reviewed in Refs. 1 and 2), bacterial acyl carrier protein (ACP) also has important functions as a donor of activated fatty acyl groups during biosynthesis of phospholipids (3), lipid A (4), lipoic acid (5), acylated homoserine lactones involved in quorum sensing (6, 7), and protein toxins, such as hemolysin (8). ACP or similar proteins are additionally involved in the synthesis of membrane-derived oligosaccharides (9), polyketide antibiotics (10), cell wall lipoteichoic acid (11), and rhizobial nodulation signaling factors (12). Despite the importance of ACP in these diverse physiological processes, very little is known about how specific amino acid residues in this small acidic protein contribute to its three-dimensional structure and interactions with various enzymes. Do different regions and residues of ACP interact with different enzymes? If so, what effect does altering ACP structure have on the multiple metabolic pathways that compete for acyl groups?

*Escherichia coli* ACP (77 amino acids, molecular weight 8847) is the prototypical type II dissociated ACP found in bacteria, plastids, and mitochondria. Two-dimensional NMR has revealed that *E. coli* ACP has a defined but flexible tertiary structure dominated by three major parallel α-helices located at residues 3–14 (I), 37–51 (II), and 65–75 (III) (13). These helices enclose a hydrophobic core along their length, providing a binding pocket for fatty acids, which are covalently attached by a thioester bond to the 4-phosphopantetheine prosthetic group located at Ser-36 (14). Fatty acid binding has little influence on ACP conformation under physiological conditions (15), but it stabilizes ACP against denaturation at alkaline pH (16–18). ACPs from spinach chloroplast (19) and *Streptomyces* type II polyketide synthase (20) exhibit tertiary structural features similar to *E. coli* ACP, indicating a remarkable degree of ACP structural conservation, which may in turn reflect constrained evolution of a protein with multiple interacting partners. Indeed, ACPs from different organisms can be functionally interchanged in some biochemical processes. Spinach and *E. coli* ACPs are utilized with similar efficiency by plant fatty acid synthases, although many corresponding *E. coli* enzymes exhibit a preference for bacterial ACP (21, 22). More distantly related ACP-like proteins can also be substrates for bacterial enzymes: the NodF factor involved in *Rhizobium* lipochitooligosaccharide synthesis is accepted by several *E. coli* fatty acid biosynthetic enzymes, but *E. coli* ACP cannot be used in reciprocal nodulation reactions (23). Although no general pattern emerges from these studies, they do indicate that some enzymes are more discriminating than others in their interactions with ACP.

An additional role of ACP is to provide myristic acid (14:0) for bioluminescence in Gram-negative marine bacteria, such as *Vibrio harveyi* (24, 25). In this process, myristic acid is diverted from further elongation to aldehyde synthesis by a luminescence-specific myristoyl-ACP thioesterase. Myristic acid may be reactivated to ACP by the soluble acyl-ACP synthetase that is present in this organism (26, 27). Recent cloning and iso-
tion of V. harveyi ACP did not reveal any features that were potentially unique to its role in bioluminescence (28). In fact, V. harveyi and E. coli ACP amino acid sequences are 86% identical, and only one of the 11 differences involves a nonconservative change in a helical region: Val instead of Gly at position 12 in V. harveyi ACP. These ACPs also share many biophysical properties (29). In the present study, we have used the V. harveyi ACP gene as a template for site-directed mutagenesis to dissect the role of individual amino acid residues in ACP structure and function, specifically those implicated in interactions with covalently attached fatty acids.

**EXPERIMENTAL PROCEDURES**

Materials—[9,10-3H]Myristic acid (49 Ci/µmol) and [α-35S]DATP (1250 Ci/µmol) were obtained from PerkinElmer Life Sciences. pGEX-5X-3 vector, T7 sequencing kit, glutathione-Sepharose 4B, and SOURCE 15Q anion exchange resin were from Amersham Pharmacia Biotech. Unlabeled fatty acids and E. coli holo-ACP were purchased from Sigma. Gel electrophoresis equipment and reagents were from Bio-Rad, whereas GelCode protein stain and Micro BCA protein assay reagents were from Pierce. Factor Xa restriction protease was obtained from Roche Molecular Biochemicals. All other chemicals used in this study were of the highest purity available.

Combination of Recombinant and Mutant V. harveyi ACP Plasmids—The V. harveyi acpP gene (GenBank™ accession number U39441 (28)) was amplified from a genomic V. harveyi library by PCR using forward (5′-AGGATCCCATGAGAACATCGAAGACGCTTAAGAA) and reverse (5′-GCTCTAGAATAGCCGCTGCTTTTGATC) primers that contained restriction sites for BamHI and XhoI (underlined), and the PCR product was ligated into the pGEX-5X-3 vector for expression as a GST fusion protein. Mutations were introduced into the coding region of ACP using the MORPH site-specific plasmid DNA mutagenesis kit (Eppendorf-5′ Inc.) and mutation specific oligonucleotides (27-33mer, Life Technologies, Inc.); all mutations were confirmed by DNA sequence analysis. Plasmids encoding GST-ACP fusion proteins were maintained in E. coli DH5α and transformed into chemically competent E. coli BL21 cells (Stratagene) for protein expression. Factor Xa cleavage of the GST-ACP product yields a recombinant V. harveyi ACP (rACP) with an N-terminal extension of four amino acids (GIPM).

Isolation and Purification of ACPs—Recombinant ACPs were produced by induction of 100 ml of mid-log phase cells (A600 = 0.5) in LB medium with 1 mg isopropyl-β-D-thiogalactopyranoside for 3 h at 30 °C. Cell pellets were suspended in 2 ml of phosphate (pH 7.0), 5 mM DTT. Fractions containing thioesterase activity were further purified by application to a SOURCE 15Q column, the amount of free thiol was calculated (six bursts of 30 s), and incubated 30 min on ice with 1% Triton X-100 prior to centrifugation (27,000 × g). The presence of the 4-kDa chain interaction, we constructed a GST-V. harveyi ACP fusion protein which provides a stable source of enzyme activity and removes endogenous ACP (26). The enzyme preparation used for determination of kinetic parameters with mutant ACPs was 1.3 mg of protein/ml and 3.5 units/mg (1 unit = 1 nmol of product formed per min under standard assay conditions (33)). Assays were performed at 37 °C in a final volume of 15 µl containing 80 µM [3H]myristic acid (800 dpm/µmol), 10 mM MgCl2, 10 mM ATP, and 1–60 µM ACP in 100 mM Tris-HCl (pH 7.8), 5 mM DTT. Acyl-ACP synthetase (2 milliunits) was added to start the reaction, and samples (10 µl) were removed at 10 min; acyl-ACP formation was measured after spotting on filter paper and washing with methanol/chloroform/acetatic acid (6/3/1, v/v) to remove unbound fatty acid (33). Blank values from reactions conducted in the absence of ACP (control) and kine were subtracted. Reaction velocities (Vmax and Km) were measured using Edac-Hofstee plots. The acyl-ACP was used under these conditions (<20% conversion of limiting ACP substrate).

Acyl-ACP synthetase was also used to prepare acyl-ACPs for native PAGE analysis and [3H]myristoyl-ACP substrates for thioesterase assay (see below), except that the reaction was allowed to proceed up to 4 h to achieve quantitative conversion to acyl-ACP (33). Samples for electrophoresis were mixed with 0.35 volumes of 4× native sample buffer (0.1 M Tris-HCl (pH 6.8), 45% (v/v) glycerol, bromphenol blue). [3H]Myristoyl-ACP substrates were further purified by application to a 1 ml DEAE-Sepharose column equilibrated in 10 mM MES, pH 6.0. After washing with buffer alone and with 50% isopropanol to remove fatty acid, [3H]Myristoyl-ACP was eluted with 0.5 µl NaCl and quantified by liquid scintillation counting.

Myristoyl-ACP Thioesterase Assay—V. harveyi myristoyl-ACP thioesterase was partially purified as described (34) with minor modifications. Briefly, cells from bright luminescent culture (650 ml, A600 = 2) were harvested and lysed by sonication in 50 ml of 50 mM sodium phosphate (pH 7.0), 5 mM DTT. The cell-free extract was subjected to ammonium sulfate fractionation, and the 30–50% precipitate was dissolved in 100 ml of the above buffer, applied to a SOURCE 15Q column, and eluted with a linear gradient (30 ml total) to 0.5 M sodium phosphate (pH 7.0), 5 mM DTT. Fractions containing thioesterase activity were identified by [3H]myristoyl-ACP cleavage (below); the 32-kDa enzyme was judged to be about 50% pure at this stage based on protein staining.

Myristoyl-ACP thioesterase activity was measured in glass tubes by incubating 0.2–1 µl of enzyme preparation with 25 nM [3H]myristoyl-ACP in 1× sodium phosphate buffer (pH 7.0) for 5 min at 25 °C. The reaction (100 µl total volume) was stopped by addition of 10 µl of acetic acid followed by extraction of released [3H]myristic acid into 1 ml of hexane (24).

**RESULTS**

**Preparation and Characterization of Mutant ACPs—** Site-directed mutagenesis is an important approach for understanding protein structure and function, but its application to acyl carrier proteins has been hampered by the toxicity of E. coli ACP when overexpressed in E. coli (31). To investigate residues involved in specific ACP functions and properties such as acyl chain interaction, we constructed a GST-V. harveyi ACP fusion protein template for alteration of amino acids by site-directed mutagenesis. V. harveyi and E. coli ACPs are among the most...
similar, characterized in terms of primary structure (86% sequence identity (28)) and hydrodynamic properties (29). Four residues that are identical in E. coli and V. harveyi ACP (Phe-50, Ile-54, Ala-59, and Tyr-71) have been previously implicated in interaction with the first 6–8 carbons of covalently attached acyl groups by NMR (14, 36) and difference spectroscopy (37). As illustrated in Fig. 1, these fatty acid binding pocket residues are located in a discrete region near the N and C termini, i.e. where they could potentially interact directly with acyl chains esterified to the phosphopantetheine attached at Ser-36 (38). All four side chains are at least partially buried in a hydrophobic core, with all but Tyr-71 potentially exposed to the acyl chain on the same face of the protein as Ser-36. These residues are also identical in 95% (Phe-50), 86% (Ile-54), 88% (Ala-59), and 62% (Tyr-71) of 56 ACP sequences examined in the nonredundant data base.

Circular dichroism was used to assess whether the N-terminal extension of four amino acids and/or mutations introduced in the fatty acid binding pocket alter the secondary structure of rACP. The far UV CD spectrum of E. coli ACP in phosphate buffer at pH 7 was consistent with previous reports indicating an α-helix content of 40–50% (39). By contrast, we were surprised to find that both V. harveyi native and rACP exhibited CD spectra more typical of random coil conformation under these conditions (Fig. 2). Schulz (39) has shown that charge neutralization by divalent cation binding, which has little effect on native E. coli ACP conformation at neutral pH, can prevent loss of secondary structure that occurs due to electrostatic repulsion either at elevated pH or upon acetylation of lysine residues at neutral pH (39). Indeed, addition of 10 mM MgCl₂ to either V. harveyi native or rACP caused a dramatic shift to a more typical native-like conformation but had little effect on E. coli ACP (Fig. 2). Thus, we conclude that V. harveyi ACP, which is slightly more acidic than the E. coli protein (28), is partially denatured at physiological pH in the absence of divalent cations.

CD analysis of the effect of Mg\(^{2+}\) on secondary structure was extended to several mutant derivatives of ACP (Fig. 3). Like V. harveyi and rACP, all fatty acid binding pocket mutants except I54A and F50A exhibited a 3-fold increase in the magnitude of [\(\theta\)]\(_{220}\) in the presence of this cation, indicating a substantial increase in helical content and native-like conformation under these conditions. A similar trend was observed for mutant V12G, which involves the nonconservative replacement of Val-12 within helix I with Gly found at the corresponding position in E. coli ACP. By contrast, the magnitude of [\(\theta\)]\(_{220}\) was lower for I54A and F50A ACPs in the absence of Mg\(^{2+}\), and this value was increased less than 1.5-fold by addition of Mg\(^{2+}\) to the sample, indicating that these mutant ACPs are incapable of adopting native conformation.

Further evidence that mutants I54A and F50A are not in a compact folded conformation even in the presence of Mg\(^{2+}\) was obtained by electrophoresis at neutral pH (Fig. 4). Under these conditions, in which recombinant and mutant ACPs should have approximately equivalent charge, most mutants exhibited identical mobility to rACP, indicating a similar hydrodynamic radius. However, the mobilities of F50A and especially I54A were decreased relative to the other proteins, suggesting that these mutant ACPs have a greater hydrodynamic radius. Mobilities of E. coli and V. harveyi ACPs were also measured by neutral pH PAGE (Fig. 4), but the results using this sensitive technique are not directly comparable to rACP and mutant derivatives due to more extensive differences in protein charge and/or size.

Acyl-ACP Synthetase Activity with Mutant ACPs—Native E. coli and V. harveyi ACPs, rACP, and several mutant derivatives were tested as substrates for V. harveyi acyl-ACP synthetase, a soluble enzyme that activates a broad range of fatty acids to acyl-ACP with hydrolysis of ATP to AMP (26). As shown in Table I, no difference in K_m was observed between rACP and the two native ACPs. Although comparison of V_max between rACP and V. harveyi ACP indicated a modest effect of the four additional N-terminal amino acids in the former, uncleaved GST-ACP was myristoylated with comparable efficiency (not shown), indicating a general tolerance of acyl-ACP synthetase to N-terminal ACP extensions. Most fatty acid binding pocket ACP mutants exhibited significantly decreased acyl-ACP synthetase efficiency relative to rACP (Table I). Increased K_m values were observed with all mutants except Y71A, for which a significantly decreased V_max was measured. V_max was also significantly decreased with mutants Y71A and I54L, and it was greatly reduced for mutant F50A, which was a very poor substrate for this enzyme. No activity was observed for I54A (but see below), and more conservative replacements at this
**Structure and Function of Acyl Carrier Protein**

Fig. 3. Effects of Mg²⁺ on secondary structure of mutant ACPs. CD spectra of the indicated ACPs (3–10 μM) were measured at 25 °C before and after addition of 10 mM MgCl₂ to samples in 10 mM sodium phosphate, pH 7.0. The magnitude of the mean residue ellipticity at 220 nm is shown; values indicate the average of 2–4 measurements, using at least two independent preparations of each protein.

![CD spectra of mutant ACPs](image)

**TABLE I**

Kinetic parameters of V. harveyi acyl-ACP synthetase with native, recombinant, and mutant ACPs

<table>
<thead>
<tr>
<th>ACP Substrate</th>
<th>Vₘₐₓ (pmol/min/μg)</th>
<th>Kᵣ (μM)</th>
<th>Relative Efficiency¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli ACP</td>
<td>3.8 ± 0.7</td>
<td>9.4 ± 4.2</td>
<td>0.92</td>
</tr>
<tr>
<td>V. harveyi ACP</td>
<td>2.3 ± 0.1</td>
<td>7.9 ± 3.0</td>
<td>0.67</td>
</tr>
<tr>
<td>rACP</td>
<td>3.8 ± 0.6</td>
<td>8.7 ± 1.6</td>
<td>(1.0)</td>
</tr>
<tr>
<td>V12G</td>
<td>2.2 ± 0.2</td>
<td>7.3 ± 0.3</td>
<td>0.69</td>
</tr>
<tr>
<td>F50A</td>
<td>0.3 ± 0.1</td>
<td>17 ± 2</td>
<td>0.04</td>
</tr>
<tr>
<td>I54A</td>
<td>2.6 ± 0.9</td>
<td>29 ± 3</td>
<td>0.20</td>
</tr>
<tr>
<td>I54L</td>
<td>4.8 ± 0.6</td>
<td>35 ± 4</td>
<td>0.31</td>
</tr>
<tr>
<td>A59G</td>
<td>3.1 ± 0.5</td>
<td>13 ± 3</td>
<td>0.54</td>
</tr>
<tr>
<td>Y71A</td>
<td>1.1 ± 0.3</td>
<td>3.0 ± 0.4</td>
<td>0.84</td>
</tr>
</tbody>
</table>

¹ Catalytic efficiency (Vₘₐₓ/Kᵣ) relative to that of rACP.

No activity, as ACP is in apo form (values not significantly above background).

**Effect of Acylation on ACP Conformational Stability—ACP is partially unfolded at pH 9, and native PAGE at this pH is known to be very sensitive to conformational changes brought about by acylation or by other modifications at Ser-36 (18). Previously, we used this method to demonstrate that acylation of V. harveyi ACP (like E. coli ACP) stabilizes the protein against alkaline pH-induced expansion and increases mobility in a chain length-dependent manner (29). As shown in Fig. 6, all unacylated mutant ACPs except Y71A exhibited a slight but measurable decrease in mobility relative to rACP, indicating a small further increase in hydrodynamic radius at pH 9. Acylation with 6:0, 10:0, and 14:0 progressively increased electrophoretic mobility of all mutant ACPs, indicating that each fatty acid binding pocket mutant is at least partially stabilized by noncovalent interaction with the acyl chain. However, acylation had significantly less effect on mobility of mutants F50A and A59G, suggesting that either the strength of acyl chain interaction or the resulting degree of compaction of the protein is decreased in these mutants.

Myristoyl-ACP Thioesterase Activity with Mutant ACPs—V. harveyi myristoyl-ACP thioesterase provides myristic acid for bioluminescence by catalyzing transfer or hydrolysis of acyl groups from either myristoyl-ACP or myristoyl-CoA (25, 34). Activity of this enzyme using acyl-ACP as a substrate is optimum at high concentrations of phosphate buffer, where it acts as a hydrodase (24). Under these conditions, all myristoyl-ACPs tested (including F50A) were effective substrates for this enzyme (Fig. 7).
Fig. 6. Electrophoretic mobility of recombinant and mutant acylated ACPs. ACPs (1 nmol) were incubated with *V. harveyi* acyl-ACP synthetase and ATP in the absence (−) or presence of the indicated fatty acid, as described in the text. Proteins were separated by conformationally sensitive PAGE at pH 9, and gels were stained for total protein; the inset shows results for rACP and the A59G mutant. Electrophoretic mobility relative to unacylated rACP is indicated, and results are representative of two similar experiments.

Fig. 7. Relative activity of *V. harveyi* myristoyl-ACP thioesterase with native, recombinant, and mutant ACPs. [3H]Myristoyl-ACPs (25 nmol) were incubated with myristoyl-ACP thioesterase (0.2 or 0.5 µl 1.8 mg of protein/ml) in 1 × sodium phosphate buffer for 5 min. ACP thioesterase cleavage was measured as described in the text and expressed as a percentage of substrate initially present. Values are the average of three assays at each enzyme concentration.

One of the more surprising results of this investigation was the discovery that ACPs derived from *V. harveyi* are unfolded at physiological pH in the absence of divalent cations. *E. coli* ACP adopts a native conformation under these conditions but is unfolded at elevated pH, at neutral pH when its four lysine residues are modified by acetylation (39), or when the first six amino acids are removed from the N terminus (40). Denaturation accompanying loss of positively charged residues can be reversed by binding of Ca^{2+} or Mg^{2+} to *E. coli* ACP, although these cations have minimal effects on the native conformation (39). Most likely, the decreased stability of *V. harveyi* relative to *E. coli* ACP at neutral pH is due to its more acidic character: *V. harveyi* ACP is predicted to have a greater negative charge (−2) in the loop region between helices I and II and also lacks a histidine residue at position 75 of *E. coli* ACP.

Like *E. coli* ACP (16, 18), both the native *V. harveyi* ACP (29) and rACP interact with fatty acids in a chain length-dependent manner to stabilize the proteins against hydrodynamic expansion at elevated pH. Identification of the residues involved in fatty acid binding has come primarily from NMR studies of *E. coli* ACP. Mayo and Prestegard (14) showed that binding of acyl chains of four carbons or longer alters chemical shifts of aromatic residues: the greatest effect was on Phe-50, whereas lesser changes attributable to minor conformational alterations were noted for Tyr-71 and other residues. Later studies demonstrated direct interaction between methyl groups of Ile-54 and Ala-59 and fluorines in 5,5-difluorohexanoyl-ACP (36). Tyr-71 was further implicated in fatty acid interaction by difference spectroscopy (37), which also suggested that this residue is in a more polar environment than Phe-50. All of these experiments are consistent with hydrodynamic data suggesting that the first 6–8 carbons of an acyl chain are sequestered by ACP, whereas more distal parts of the chain are exposed and increase hydrophobicity of the protein (17).

As the amino acid sequences of *E. coli* and *V. harveyi* ACPs are identical between residues 31 and 71 (28), we would predict that the above residues are also involved in acyl chain interaction in *V. harveyi* ACP. Indeed, gel filtration did not reveal any differences in Stokes radius between *E. coli* and *V. harveyi* acyl-ACPs, although the more sensitive native PAGE method indicated a more pronounced effect of fatty acids on the latter protein (29). Native PAGE has been used in the present study to show that all ACP mutants that can be acylated have at least some ability to interact noncovalently with acyl groups and stabilize the protein in a chain length-dependent manner. This ability was most affected by mutation of Ala-59 or Phe-50, two residues that are exposed in the fatty acid binding pocket but are not in very close proximity in *E. coli* ACP (Fig. 1). In the case of A59G, stabilization was decreased without global conformational change, suggesting that this residue may interact directly with the fatty acyl chain, as reported previously (36). Decreased acyl chain stabilization in mutant F50A is more likely to be an indirect effect caused by extensive conformational disruption of this ACP, although increased mobility with longer chain fatty acids indicates some residual interaction. Like Ala-59, Ile-54 has been shown to interact directly with fatty acids (36), and this interaction would appear to be largely retained in the conservative I54L and I54V mutants. The Y71A mutation also had little effect on PAGE mobility or its decrease upon acylation, perhaps consistent with its more polar environment (37) not directly in contact with the acyl binding groove of ACP (Fig. 1). Note that Tyr-71 is also less conserved among ACPs than other residues examined here, and a recent study has indicated that dansylation of *E. coli* ACP at this position does not abolish its effectiveness as a substrate for ACP synthase, acyl-ACP synthetase, or Δ9-desaturase (43).
Kinetic analysis of V. harveyi acyl-ACP synthetase with a variety of mutant ACPs indicates that this enzyme prefers ACP in its native conformation and may interact with residues in the fatty acid binding pocket. Very little activity was obtained with mutant F50A (4% catalytic efficiency of rACP), although the partially unfolded character of this ACP precludes assignment of a direct role in acyl-ACP synthetase activity. On the other hand, the elevated \( K_m \) with mutants I54V and I54L, which appear to be in a native-like conformation, does suggest involvement of Ile-54 in interaction with the enzyme. Mutation of Ala-59, a residue that plays a role in acyl chain binding (above), had relatively little influence on acyl-ACP synthetase activity. Most interesting is mutant Y71A, with which the enzyme exhibited little decrease in catalytic efficiency, but an unusual decrease in \( K_m \). More replacements at this position will be necessary to interpret the role of this residue in acyl-ACP synthetase activity. Finally, mutation of Val-12 to Ala at a site that had no effect on the activity of acyl-ACP synthetase, myristoyl-CoA (34) and myristoyl-ACP thioesterase (46), whereas acyl-ACP synthetase is constitutively induced during bioluminescence development of V. harveyi. It also supports the partially unfolded character of this ACP precludes assignment of a direct role in acyl-ACP synthetase activity. On the other hand, the elevated \( K_m \) with mutants I54V and I54L, which appear to be in a native-like conformation, does suggest involvement of Ile-54 in interaction with the enzyme. Mutation of Ala-59, a residue that plays a role in acyl chain binding (above), had relatively little influence on acyl-ACP synthetase activity. Most interesting is mutant Y71A, with which the enzyme exhibited little decrease in catalytic efficiency, but an unusual decrease in \( K_m \). More replacements at this position will be necessary to interpret the role of this residue in acyl-ACP synthetase activity. Finally, mutation of Val-12 to Ala at a site that had no effect on the activity of acyl-ACP synthetase, myristoyl-CoA (34) and myristoyl-ACP thioesterase (46), whereas acyl-ACP synthetase is constitutively induced during bioluminescence development of V. harveyi.
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