Mutational Analysis of a Fatty Acyl-Coenzyme A Synthetase Signature Motif Identifies Seven Amino Acid Residues That Modulate Fatty Acid Substrate Specificity*

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Paul N. Black‡, Qing Zhang§, James D. Weimar, and Concetta C. DiRusso
From the Department of Biochemistry and Molecular Biology, The Albany Medical College, Albany, New York 12208

Fatty acyl-CoA synthetase (fatty acid:CoA ligase, AMP-forming; EC 6.2.1.3) catalyzes the formation of fatty acyl-CoA by a two-step process that proceeds through the hydrolysis of pyrophosphate. In Escherichia coli this enzyme plays a pivotal role in the uptake of long chain fatty acids (C12–C18) and in the regulation of the global transcriptional regulator FadR. The E. coli fatty acyl-CoA synthetase has remarkable amino acid similarities and identities to the family of both prokaryotic and eukaryotic fatty acyl-CoA synthetases, indicating a common ancestry. Most notable in this regard is a 25-amino acid consensus sequence, DWGLHTGIDGXMWXXPXGKLHIDRRK, common to all fatty acyl-CoA synthetases for which sequence information is available.

Within this consensus are 8 invariant and 13 highly conserved amino acid residues in the 12 fatty acyl-CoA synthetases compared. We propose that this sequence represents the fatty acyl-CoA synthetase signature motif (FACS signature motif). This region of fatty acyl-CoA synthetase from E. coli, 43NGWGLHTGIDAVMDEEGFLRIVDRKK, contains 17 amino acid residues that are either identical or highly conserved to the FACS signature motif. Eighteen site-directed mutations within the fatty acyl-CoA synthetase structural gene (fadD) corresponding to this motif were constructed to evaluate the contribution of this region of the enzyme to catalytic activity. Three distinct classes of mutations were identified on the basis of growth characteristics on fatty acids, enzymatic activities using cell extracts, and studies using purified wild-type and mutant forms of the enzyme: 1) those that resulted in either wild-type or nearly wild-type fatty acyl-CoA synthetase activity profiles; 2) those that had little or no enzyme activity; and 3) those that resulted in lowering and altering fatty acid chain length specificity. Among the 18 mutants characterized, 7 fall in the third class. We propose that the FACS signature motif is essential for catalytic activity and functions in part to promote fatty acid chain length specificity and thus may compose part of the fatty acid binding site within the enzyme.

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‡ An established investigator of the American Heart Association. To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, The Albany Medical College, 47 New Scotland Ave., Albany, NY 12208. Tel.: 518-262-6416; Fax: 518-262-5689.
§ Current Address: Dept. of Pediatrics, University of Tennessee College of Medicine, 800 Madison Ave., Memphis, TN 38163.

1 C. C. DiRusso, unpublished observations.
that the synthetase is a component of a fatty acid transport apparatus in agreement with recent data showing that the transport and activation of exogenous long-chain fatty acids in mouse adipocytes occurs via a fatty acid transport protein and associated fatty acyl-CoA synthetase (23).

The E. coli fatty acyl-CoA synthetase structural gene (fadD) has been cloned and sequenced and shown to encode a protein of 561 amino acid residues (26, 27). This enzyme shares amino acid sequence similarities to other fatty acyl-CoA synthetases and more broadly the family of adenylate-forming enzymes (28–29). The placement of this enzyme in the family of adenylate-forming enzymes is based on amino acid similarities within a proposed AMP/ATP binding domain (25). Of particular relevance to the present work was the identification of a second conserved region that appears to be restricted to the family of fatty acyl-CoA synthetases (26). Within this latter region is a highly conserved 25-amino acid residue segment that we propose specifies a signature motif common to the family of fatty acyl-CoA synthetases. A series of site-directed alanine substitutions within the fadD gene corresponding to this signature motif were constructed and analyzed to provide information regarding the role of this region to the catalytic activity of the enzyme. Our present data confirmed the prediction that this region of the enzyme was essential for activity. Three distinct classes of mutations were identified on the basis of growth characteristics of a fadD strain harboring these alleles on a low copy plasmid, fatty acyl-CoA synthetase profiles using oleate and decanoate as substrates, and studies using purified mutant forms of the enzyme: 1) those that resulted in either wild-type or nearly wild-type fatty acyl-CoA synthetase activity profiles; 2) those that had little or no enzyme activity; and 3) those that resulted in altered fatty acid chain length specificity.

Within this latter group, seven mutant fadD alleles have been generated that have altered activity with fatty acids of differing chain lengths when compared with the wild type. On the basis of the data presented in this work, we propose that this signature motif within fatty acyl-CoA synthetase functions in part to promote fatty acid chain length specificity and may compose part of the fatty acid binding site.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions**—The bacterial strains used in this study were BH407 (thi supE xylo proAB) mutS::Tn10 (F' proAB lacZAM15); JM109 (endA1 relA1 gyrA96 thi hsdR17 (rK- mK-)) relA supE44 λ (ΔlacproAB) (F' trd36 proAB lacZAM15); LS1187 (C600 xyfAD); LS9928 (fadR fadD88 sacA::Tn10); and BL21 (DE3)pLysSs (8, 29–31). Bacterial cultures were grown at 37°C in a Lab Line gyratory shaker in Luria broth (LB) or terrific broth (TB). When minimal media were required, medium E supplemented with vitamin B<sub>12</sub> was used (32). Carbon sources, sterilized separately, were added to final concentrations of 25 mM glucose, 25 mM potassium acetate, 5 mM decanoate, or 5 mM oleate. When oleate or decanoate was used as a carbon source, poloxymethylene 20 cetol ether (Brij 58) was added to a final concentration of 0.01%. When required to maintain plasmids, antibiotics were added to 100 μg/ml ampicillin, 40 μg/ml kanamycin, 10 μg/ml tetracycline, and 40 μg/ml chloramphenicol. Growth of bacterial cultures was routinely monitored using a Klett-Summerson<sup>TM</sup> colorimeter equipped with a blue filter.

The plasmids used in this study are listed in Tables I and II. Details concerning the construction of the plasmids harboring the fadD mutants are described below and under "Results."

**Sequence Comparisons Using Biological Sequence Comparative Analysis Node (BioSCAN)**—Protein sequence comparisons were performed using BioSCAN<sup>2</sup> with sequences that are conserved among the fatty acyl-CoA synthetases. These comparisons were directed against the SWISS-PROT data base using a 0.01 probability threshold with complexity filtering. The score table employed was bloom62.

**General Recombinant DNA Methods**—Restriction, ligation, and transformation procedures have been previously described (33). Oligonucleotides (17–24-mer, for mutagenesis and sequencing) were synthesized on a Pharmacia Biotech Inc. Gene Assembler Plus and purified by phenol-chloroform extraction and ethanol precipitation. The final concentrations of oligonucleotides were estimated by determination of optical density at 260 nm. When required, oligonucleotides (100 pmol) were phosphorylated using 5 units of T4 polynucleotide kinase in 50 mM Tris-HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 5 mM dithiothreitol, 0.1 mM spermidine, 1 mM ATP. Small scale plasmid isolation was achieved by using alkaline lysis (33) or by using QiaPrep columns as specified by the vendor (Qiagen). Single-stranded phagemid DNA and plasmid DNA were isolated from the different mutants and sequenced using the chain termination method of Sanger et al. (34) using [α-<sup>35</sup>S]dATP and Sequenase version 2.0 (U.S. Biochemical Corp.). Synthetic oligonucleotides (17- and 21-mer) complimentary to various regions of the fadD gene were used as primers (26).

**Construction of Deletion and Site-directed Mutations in fadD**—Site-directed mutations in the fadD gene were generated using the Altered Sites<sup>TM</sup> mutagenesis system of Promega. The EcoRl-HinDIII fragment from pN324 (26) was gel-purified and ligated into the phagemid pSLECT<sup>TM</sup> to generate pN351. This phagemid contained the entire fadD sequence. A limited number of phagemid clones were randomly selected, and the entire coding sequences of the fadD and fadR genes in opposite orientations. fadR was included in these constructs to prevent toxicity of fadD expression in this high copy plasmid. Phosphorylated mutagenic oligonucleotides harboring the required mutation (1–2 pmol; see Table I) and the ampicillin repair oligonucleotide (0.25 pmol) were annealed to single-stranded pN351 phagend DNA (100–250 ng) in 20 mM Tris-HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 50 mM NaCl by heating at 70°C for 5 min followed by slowly cooling to room temperature. Following annealing, the mutagenic oligonucleotides were extended with T4 DNA polymerase (10 units) and ligated with T4 DNA ligase (2 units) in 10 mM Tris-HCl, pH 7.5, 1 mM ATP, 2 mM dithiothreitol, and 0.5 mM dNTPs (dATP, dGTP, dTTP, and dCTP). The double-stranded DNA was transformed into a repair-defective strain of E. coli (BMH 71–18) using the procedures described below and under "Results."

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2 The abbreviations used are: LB, Luria broth; TB, terrific broth; FACS, fatty acyl-CoA synthetase.

3 Available from the University of North Carolina on the World Wide Web at http://genome.cs.unc.edu/prot-match.html (Ref. 50).
Fatty Acyl-CoA Synthetase Signature Motif

Materials—[1-3H]Oleic acid, [1-3H]Myristate, [2-3H]Methionine, and leucine were purchased from Du Pont NEN. [1-14C]Decanoic acid was purchased from Sigma. Enzymes for DNA sequencing (Sequenase) were obtained from U.S. Biochemical Corp., and enzymes for routine DNA manipulations were obtained from Life Technologies, Inc., Pharmacia, U.S. Biochemical Corp., or New England Biolabs. Chemicals for the synthesis of fadD-specific oligonucleotides and the mutagenic oligonucleotides were purchased from ABN/Biogenex or Pharmacia. Antibiotics and other supplements for bacterial growth were obtained from Difco and Sigma. All other chemicals were obtained from standard suppliers and were of reagent grade.

RESULTS

Comparison of Adenylate-forming Enzymes Using Biological Sequence Comparative Analysis Node (BioSCAN)—In our previous work we identified two regions within the E. coli fatty acyl-CoA synthetase that were similar to other adenylate-forming enzymes (24). The first region, corresponding to amino acids 200–273 within the E. coli enzyme, contains a putative AMP binding site. On the basis of this highly conserved sequence common to all adenylate-forming enzymes, the E. coli fatty acyl-CoA synthetase has been placed into the superfamily of AMP-binding proteins (27). This region contains a sequence that is similar to ATP-binding P-loops and may represent part of the ATP binding pocket within fatty acyl-CoA synthetase (37, 38). The second region that we noted having similarity to other adenylate-forming enzymes corresponds to amino acid residues 353–455 of the bacterial enzyme. Using BioSCAN, we established the methods. The pool of transformed cells was grown in LB containing 100 μg/ml ampicillin followed by phagemid DNA isolation. Phagemid DNA pools were transformed into strain JM109 on selection on LB plates containing 100 μg/ml ampicillin. Phagemid DNA was isolated from individual colonies as recommended by Promega. The collection of specific promoter. Expression plasmids containing specific fusD alleles mutation were gel-purified and ligated into pACYC177. These final plasmid constructions are listed in Table I, and upon transformation into the appropriate host strains were analyzed as detailed below.

Construction of Amino-terminal Histidine-tagged Fatty Acyl-CoA Synthetase—An EcoRI site was generated at the +1 site of translation of the E. coli fatty acyl-CoA synthetase in the phagemid pN351 to generate pN3571. The EcoRI-HindIII fragment from pN3571 containing the coding region of the fusD gene was gel-purified and ligated into pRSET-B (Invitrogen) to generate pN3576. The expression plasmid pN3576 contains six histidine residues and an enterokinase cleavage site preceding the coding sequence of the fusD gene. Expression of His-tagged fatty acyl-CoA synthetase is driven off a T7 RNA polymerase responsive promoter. Expression plasmids containing specific fusD mutations were generated by ligating gel-purified SulI-HindIIIII fragments from the phagemid constructs listed in Table I into pN3576 digested with SulI and HindIII. The final designations of the expressed mutants are listed in Table II.

Overexpression of Amino-terminal Hexameric Histidine-tagged Fatty Acyl-CoA Synthetase—The plasmids listed in Table II were transformed into strain BL21 (DE3) (pLysS), and wild-type or mutant forms of the enzyme were overexpressed following induction of midlogarithmic growth in either LB or minimal medium supplemented with isopropyl-β-D-thiogalactopyranoside for 90 min (31). Following induction, cells were harvested and prepared as described below. For unfractionated enzyme purification or resuspended in SDS sample buffer and boiled, and proteins were resolved on a 9 or 12% SDS-polyacrylamide gel using the Laemmli buffer system (35). Cells that were grown in minimal media were treated with rifampicin for the last 60-min period of induction as described previously (24) and labeled with [35S]methionine. In this case, samples were prepared as described above, and following electrophoresis, the gels were dried and subjected to autoradiography for 2–24 h.

Purification of Histidine-tagged Fatty Acyl-CoA Synthetase—The bacterial strain BL21 (DE3) (pLysS) containing the different expression plasmids listed in Table II were grown to midlog phase (6 × 109 cells/ml) in 30–50-ml cultures of LB containing ampicillin and chloramphenicol. Following induction of T7 RNA polymerase, supplements for bacterial growth were added to the cells, harvested, and prepared as described below for enzyme purification or resuspended in SDS sample buffer that had been previously equilibrated with buffer A. The cell extract-Ni2+-NTA-agarose slurry was incubated for 2–12 h at 4 °C with end-over-end rotation and finally applied to a 0.5-cm (inner diameter) column. The column was washed with 30 ml of buffer A; 30 ml of 50 mM sodium phosphate, pH 6.0, 300 mM NaCl, 10% glycerol (buffer B) containing 50 mM imidazole; and 5 ml of buffer B containing 100 mM imidazole. The His-tagged enzyme was eluted from the column using a imidazole step gradient (750 μl of 50 mM, 200 mM, 250 mM, 300 mM, 400 mM, 500 mM, and 600 mM imidazole in buffer B). Purification was monitored on 9% SDS-polyacrylamide gels using the Laemmli buffer system (35). The His-tagged fatty acyl-CoA synthetase generally eluted between 200 and 300 mM imidazole from the Ni2+-NTA-agarose column and was greater than 95% pure. The purified His-tagged enzyme was dialyzed against buffer B, ATP was added to a final concentration of 50 mM, and the sample was frozen at ~80 °C in 100–500-μl aliquots.

Fatty acyl-CoA synthetase activity was stable in the frozen enzyme over a period of at least 4 months. The mutant forms of the enzyme over a period of at least 4 months. The mutant forms of the enzyme showed no obvious change in their properties over the period of study.

Analysis of Fatty Acyl-CoA Synthetase Activity—Bacteria (wild-type and fusD strains containing the collection of fusD+ and fusD clones) were grown to midlog phase (6 × 109 cells/ml) in TB or TB supplemented with 5 mM oleate and 0.5% Brij 58 (TBO) and with antibiotics as required. Cells were harvested by centrifugation, washed twice with medium E, resuspended to a density of 1.2 × 109 cells/ml in 10 mM Tris-HCl, pH 7.5, and lysed by three cycles of sonication at 0 °C. Fatty acyl-CoA synthetase activities were determined in sonicated cell extracts or using the purified wild-type or mutant hexameric histidine-tagged enzymes as described by Kameda and Nunn (24). The reaction mixtures contained 200 μM Tris-HCl, pH 7.5, 2.5 mM ATP, 8 mM MgCl2, 2 mM EDTA, 20 mM NaF, 0.1% Triton X-100, 10 μM [1-3H]oleate, [1-3H]myristate, [2-3H]methionine, and [1-14C]decanoate in a total volume of 0.5 ml. The reactions were initiated with the addition of coenzyme A, incubated at 35 °C for 10 min, and terminated by the addition of 2.5 ml of isopropyl alcohol, n-heptane, 1 ml H2SO4 (40:10:1). The radioactive fatty acid was removed by organic extraction using n-heptane. Fatty acyl-CoA formed during the reaction remained in the aqueous fraction and was quantified by scintillation counting. Protein concentrations in the enzyme extracts and purified enzyme samples were determined using the Bradford assay and bovine serum albumin as a standard (36). The values presented represent the average from at least three independent experiments. All experiments were analyzed using analysis of variance (StatView; Abacus Concepts, Inc.)

Fatty Acid Binding—Fatty acid binding capacity of the wild-type and mutant forms of fatty acyl-CoA synthetase were determined using Lipidex (for C18:1) or LH-20 (C10:0) as described previously (17). For oleic acid binding, substrate concentration was varied from 10 nM to 2.5 μM while the purified enzyme concentration was held at 0.2 mg/ml in 200 mM Tris-HCl, pH 7.5. Samples were incubated for 20 min at 37 °C, cooled to 0 °C in a salt-ice bath for 5 min, centrifuged (30 s at 5,000 rpm) through a 5% sucrose cushion, and resuspended in buffer, and held at 0 °C. The amount of protein-bound fatty acid in the eluate was determined by scintillation counting. For deanoic acid binding, the substrate concentration was varied from 50 nM to 50 μM while the purified enzyme concentration was held at 0.2 mg/ml. Samples were treated as above, but they were passed through LH-20 instead of Lipidex. The amount of protein-bound fatty acid in the eluate was determined by scintillation counting. Background counts from samples containing no protein were subtracted from those in the eluates containing the protein samples. The data were converted into nmoles of fatty acid bound/mg of purified protein. All of the data presented represent the average from at least three independent experiments.
addition to the fatty acyl-CoA synthetases, the more distantly related enzymes, 4-coumarate-CoA ligase (EC 6.2.1.12) and Photinus-luciferin 4-monooxygenase (EC 1.13.12.7; flyれば luciferae), share similarities with this motif. It is noteworthy that the coumarate-CoA ligases and luciferases lack both the carboxyl group of the aspartate does not contribute to the catalytic activity of this enzyme.

Four fadD alleles resulted in depressing fatty acyl-CoA synthetase to 30–50% of wild-type levels but had no change in fatty acid chain length specificity (fadDG432A, fadDH435A, fadDG437A, and fadDI450A). Included within this group are two of the highly conserved glycine residues within the FACS signature motif. In both of these cases, alanine substitutions were insufficient to completely disrupt activity. Three fadD alleles resulted in fatty acyl-CoA synthetase activities that were comparable with the fadD null (fadDW433A, fadDT436A, and fadDR453A). Three additional substitutions resulted in nearly abolishing both decanoyl-CoA and oleoyl-CoA synthetase activities but retaining detectable myristoyl-CoA synthetase activities (fadDL434A, fadDD438A, and fadDG446A). These data argue that either these residues are crucial for catalytic activity or these substitutions disrupted protein structure to the point that the mutant forms of the enzymes were either dysfunctional or nearly dysfunctional.

The alleles fadDI439A, fadDL448A, and fadDR449A resulted in lowering fatty acyl-CoA synthetase activity for all three substrates but had a change in specificity. These mutations resulted in a higher oleoyl-CoA synthetase activity relative to the decanoyl-CoA synthetase activity. Just downstream, the alleles fadDV451A, fadDD452A, fadDK454A, and fadDK455A had high decanoyl-CoA synthetase activities relative to oleoyl-CoA synthetase activities. As noted above, the fadDK454A and fadDK455A alleles resulted in an increased growth rate on decanoyl when compared with the wild type. The finding that specific amino acid substitutions within the FACS signature motif change substrate specificity is consistent with the proposal that this region of the enzyme is directly involved in fatty acid binding.
acyl-CoA synthetase so that the enzyme could be rapidly purified using a nickel chelate affinity chromatography following expression from a T7 RNA polymerase-responsive promoter. Using this construct, we were able to purify substantial quantities of His6-tagged fatty acyl-CoA synthetase in a single-step purification protocol (Fig. 3A). The induction of the T7 RNA polymerase resulted in a 1800-fold increase in enzyme activity using [3H]oleate as a substrate. Using the purified His-tagged enzyme, we demonstrated that at different concentrations of enzyme, the production of fatty acyl-CoA was linear over a 30-min period. Furthermore, the apparent \( V_{\text{max}} \) and \( K_m \) using oleate as a substrate were estimated to be 292 nmol/min/mg protein and 2.2 \( \mu \)M, respectively. The \( K_m \) was slightly lower than that defined for the purified enzyme, while the apparent \( V_{\text{max}} \) was quite similar to that of the purified enzyme (24). Consistent with our data showing fatty acyl-CoA synthetase activities in the \( \text{fad}D \) \( \text{fadR} \) strain LS6829 harboring pN300 (\( \text{fadD}^{-} \)), the purified enzyme had maximal specificity for myristate (C14:0), which is also in agreement with the purified enzyme (24, 25).

**Discussion**

*E. coli* contains a single fatty acyl-CoA synthetase with broad chain length specificity for saturated, unsaturated, and polyunsaturated fatty acids (24, 25). This enzyme is essential for the activation of exogenous long chain fatty acids destined for \( \beta \)-oxidation and plays an essential role in the regulation of the transcription factor FadR. We and others (26–28) have demonstrated that the *E. coli* fatty acyl-CoA synthetase shares considerable similarities with other fatty acyl-CoA synthetases.
and more broadly, the superfamily of adenylate-forming enzymes. The present study was undertaken to evaluate the functional role of a 103-amino acid residue segment within the *E. coli* fatty acyl-CoA synthetase that is common to other fatty acyl-CoA synthetases and several members of the AMP-binding protein superfamily. Using BioSCAN, we have identified a stretch of 25 amino acid residues within this 103-amino acid residue segment that appears to be restricted to the family of fatty acyl-CoA synthetases. We propose that the consensus sequence, DGWLHTGDIGXWXXPGXLKIIDRKK, is common to all fatty acyl-CoA synthetases and represents a FACS signature motif.

There are a number of features within the FACS signature motif that are notable. 1) This region contains two invariant glycine residues (at positions 2 and 7) and a highly conserved (11/12) glycine at position 16. Therefore, it is reasonable to predict that this region in all fatty acyl-CoA synthetases adopts a similar tertiary structure. 2) This region contains an additional six residues that are invariant in the family of fatty acyl-CoA synthetases: Trp at position 3, Thr at position 6, Asp at position 8, Asp at position 22, Arg at position 23, and Lys at position 25. 3) The consensus sequence predicts an aspartic acid residue at position 1; however, in the bacterial enzyme this is an asparagine, and conversion of the asparagine to alanine has no effect on enzyme activity, indicating that the presence of the carboxylate is not crucial for activity. 4) The residue in the fourth position is hydrophobic and is a leucine (6/12), a methi-
of the fatty acid and thus be crucial for the formation of the adenylate intermediate. We cannot conclusively distinguish these possibilities at the present time.

The analyses of the His-tagged mutant enzyme forms confirmed the data generated in whole cell extracts. Both the fadDD452A and fadDK455A mutations had high decanoyl-CoA synthetase activity relative to oleyl-CoA synthetase activity. In addition, both fadDW433A and fadDD438A had no fatty acyl-CoA synthetase activity, arguing that these Trp433 and Asp438 are essential for function. At this point, we cannot discern the precise step in the catalytic cycle that is disrupted in these two mutants. The fatty acid binding profiles obtained further support the conclusion that this region of fatty acyl-CoA synthetase is involved in fatty acid binding.

As mentioned under “Results,” both the coumarate CoA ligases and firefly luciferases contain regions that share amino acid similarities to the FACS signature motif. The substrates for these enzymes, coumarate and firefly luciferin, are hydrophobic cyclic compounds that form adenylated intermediates as part of the reaction mechanism. In this regard, coumarate CoA ligase and firefly luciferase have catalytic mechanisms similar to the fatty acyl-CoA synthetases. These two enzymes deviate from the FACS signature in that each contains an aspartate or a lysine in lieu of a glycine at position 16 and a leucine instead of a lysine at position 24. Additionally, these two enzymes contain either a phenylalanine or a tyrosine at position 11 of the motif. The glycine residue at position 16 is present in all members of the fatty acyl-CoA synthetase family with the exception of the presumed fatty acyl-CoA synthetase from Helicobacter pylori. Perhaps this glycine contributes to protein structure by maintaining a fatty acid binding pocket that cannot accommodate the cyclic coumarate or luciferin. As noted above, two fatty acyl-CoA synthetases with medium chain specificity have an aliphatic amino acid in place of a basic amino acid at position 24. Both the coumarate CoA ligases and luciferases contain a leucine at position 24, suggesting that these two enzymes are more similar to those fatty acyl-CoA synthetases with a medium chain length specificity. Coumarate CoA ligase and luciferase also contain either a phenylalanine or a tyrosine at position 11. Of the fatty acyl-CoA synthetases, only Faa2p from yeast contains a phenylalanine at this position. Faa2p has a preference for medium chain substrates. Thus, if the presumption is correct that the FACS signature motif is involved in the recognition of fatty acid substrates, small changes within this region are expected to have considerable changes on substrate recognition. This may result in preferences for hydrophobic cyclic substrates as opposed to long or medium chain alkyl substrates.

A number of enzymes that form adenylated intermediates were not detected using the FACS signature motif as a query sequence against the nonredundant data base. We and others have noted that there exists a second region of homology within the family of fatty acyl-CoA synthetases that also includes most adenylate-forming enzymes. Most notable in this regard is a region of the E. coli enzyme from amino acid residues 200–273. This region contains a signature that is presumed to specify AMP binding (26–28, 49). From the data described in the present work, it is tempting to speculate that adenylate-forming enzymes are of common ancestry. The family of the fatty acyl-CoA synthetases have maintained that AMP binding signature even evolving their own specific properties to include a presumed fatty acid binding domain that is typified by the FACS signature motif.

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