Affinity Labeling Fatty Acyl-CoA Synthetase with 9-p-Azidophenoxy Nonanoic Acid and the Identification of the Fatty Acid-binding Site*

Paul N. Black‡‡, Concetta C. DiRusso‡, David Sherin‡, Robert MacColl‡, Jens Knudsen¶, and James D. Weimar‡

From the ‡Center for Cardiovascular Sciences, Albany Medical College, Albany, New York 12208, the ¶Division of Molecular Medicine, Wadsworth Center, Albany, New York 12201, and the ¤Biochemistry and Molecular Biology Institute, University of Southern Denmark, Odense M, Denmark

Fatty acyl-CoA synthetase (FACS, fatty acid-CoA ligase, AMP-forming, EC 6.2.1.3) catalyzes the esterification of fatty acids to CoA thioesters for further metabolism and is hypothesized to play a pivotal role in the coupled transport and activation of exogenous long-chain fatty acids in Escherichia coli. Previous work on the bacterial enzyme identified a highly conserved region (FACS signature motif) common to long- and medium-chain acyl-CoA synthetases, which appears to contribute to the fatty acid binding pocket. In an effort to further define the fatty acid-binding domain within this enzyme, we employed the affinity labeled long-chain fatty acid [3H]9-p-azidophenoxy nonanoic acid (APNA) to specifically modify the E. coli FACS. [3H]APNA labeling of the purified enzyme was saturable and specific for long-chain fatty acids as shown by the inhibition of modification with increasing concentrations of palmitate. The site of APNA modification was identified by digestion of [3H]APNA cross-linked FACS with trypsin and separation and purification of the resultant peptides using reverse phase high performance liquid chromatography. One specific [3H]-labeled peptide, T33, was identified and following purification subjected to NH2-terminal sequence analysis. This approach yielded the peptide sequence PDATDEIK, which corresponded to residues 422 to 430 of FACS. This peptide is immediately adjacent to the region of the enzyme that contains the FACS signature motif (residues 431–455). This work represents the first direct identification of the carboxyl-containing substrate-binding domain within the adenylate-forming family of enzymes. The structural model for the E. coli FACS predicts this motif lies within a cleft separating two distinct domains of the enzyme and is adjacent to a region that contains the AMP/ATP signature motif, which together are likely to represent the catalytic core of the enzyme.

Over the past 15 years, there has been increasing interest in the roles played by bioactive lipids in cellular homeostasis, including fatty acids and fatty acid derivatives. It is now widely accepted that these compounds influence a wide variety of cellular processes including phospholipid synthesis (1, 2), protein export (3), protein modification (4–6), enzyme activation or deactivation (7–10), cell signaling (11, 12), membrane permeability (13), membrane fusion (14), and transcriptional control (15–19). Our interest in bioactive lipids stems from investigations directed at elucidating the mechanisms governing how exogenous long-chain fatty acids traverse the membrane, become activated to CoA thioesters and influence downstream events using both bacterial and yeast model systems (20, 21). It is recognized that fatty acids traverse the cell envelope of Escherichia coli by a protein-mediated process involving both FadL (an outer membrane-bound long-chain fatty acid transport protein) and fatty acyl-CoA synthetase (FACS); fatty acid: CoA ligase, AMP forming; EC 6.2.1.3). The formation of the fatty acyl-CoA thioester by FACS renders this process unidirectional and thus the role this enzyme plays in transport has been described as vectorial esterification (22). Similarly in yeast at least three fatty acyl-CoA synthetases have been implicated as components of the fatty acid import apparatus (23, 24).

Fatty acyl-CoA synthetase plays a central role in intermediary metabolism by catalyzing the formation of fatty acyl-CoA by a two-step process proceeding through an adenylated intermediate. In E. coli, the specificity of this enzyme is primarily directed toward long-chain fatty acids (C14:0–C18:1), but it is clear this enzyme can also activate medium-chain fatty acids (C8:0–C12:0) (25). In previous work (26, 27), we characterized the FACS structural gene, fadD, and using alanine-scanning mutagenesis, identified a region of the enzyme hypothesized to contribute to the fatty acid-binding site (FACS signature motif). The FACS signature motif is common to both eukaryotic and prokaryotic fatty acyl-CoA synthetases indicating that it is important to the structure and/or catalytic efficiency of these enzymes. This sequence motif is present in acyl-CoA synthetases with medium- to long-chain specificity and not found in the very long-chain acyl-CoA synthetases or acetyl-CoA synthetases suggesting it is fairly restrictive. Our studies identified specific mutations within fadD corresponding to the FACS signature motif that resulted in altering the chain length specificity of the enzyme. More specifically, we were able to generate altered forms of FACS that preferred medium-chain fatty acids as substrates as opposed to long-chain fatty acids (27). In this regard, we hypothesized this region of FACS is involved in fatty acid binding and provides a molecular ruler to specify chain length (27).

The E. coli FACS is a central component of the long-chain

* This work was supported by National Science Foundation Grant MCB-9816414. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Center for Cardiovascular Sciences, The Albany Medical College, 47 New Scotland Ave., MC-8, Albany, NY 12208. Tel.: 518-262-6416; Fax: 518-262-8101; E-mail: blackp@mail.amc.edu.

1 The abbreviations used are: FACS, fatty acyl-CoA synthetase; HPLC, high performance liquid chromatography; APNA, [3H]9-p-azidophenoxy nonanoic acid.

2 N. J. Færgeman, P. N. Black, and C. C. DiRusso, unpublished data.
fatty acid transport apparatus. Once transported across the cell envelope and activated to CoA thioesters, long-chain fatty acids are primarily degraded via \( \beta \)-oxidation but can be incorporated into phospholipids. In addition, exogenously derived long-chain fatty acyl-CoA acts as potent bioactive lipids by specifically regulating the DNA binding activity of the transcription factor FadR (15, 18). When the intracellular levels of long-chain fatty acyl-CoA are low, FadR is DNA bound and acts both to repress the transcription of genes involved in fatty acid transport, activation, and \( \beta \)-oxidation (faD) and to activate the transcription of at least two genes required for fatty acid biosynthesis (faB) (18, 28). FadR specifically binds long-chain fatty acyl-CoA as their intracellular level increase, which results in a loss of DNA binding activity. The net result is the derepression of the faD genes and a decrease in the expression of the faB genes. These observations support the notion that FadR responds to the intracellular pools of long-chain fatty acyl-CoA to coordinate the differential expression of genes involved in fatty acid biosynthesis and degradation.

In the present work, we have employed a direct biochemical approach using affinity labeling to probe the fatty acid-binding domain within purified \( E. coli \) FACS. These experimental methods identified a peptide containing the affinity labeled fatty acid that begins at Pro\(^{422}\) and contains the FACS signature motif supporting the proposal that this region of the enzyme represents the fatty acid-binding domain. These data, in combination with computer modeling to predict the structure of FACS and our past work using alanine-scanning mutagenesis, are in agreement with the hypothesis that the fatty acid and ATP-binding sites within the enzyme are localized within a cleft separating two distinct domains of the enzyme.

**EXPERIMENTAL PROCEDURES**

**His\(_6\)-FACS Overexpression and Purification—** Strain BL21 (ADE3::lyS8) was transformed with plasmid pN3576 encoding His\(_6\)-FACS. The expression and purification of His\(_6\)-FACS has been previously described (27). Briefly, cells (600-mL cultures) were induced with isopropyl-1-thio-\( \beta \)-D-galactopyranoside (0.5 mM) for 90 min and then purified from a clarified cell extract using Ni\(^2+\)-charged sepharose Blue, soaked in En\(^{3H}\)ance (PerkinElmer Life Sciences), dried, and subjected to fluorography for 5–7 days at \(-80^\circ\)C. For the experiments showing specificity of labeling, 400-pmol aliquots of FACS (using the buffering conditions defined above) were incubated with 1500 pmoles of \( ^{3H} \)APNA and increasing concentrations of lignocerate (C24:0), palmitate (C16:0),olenate (C18:0), hexanoate (C6:0), or acetate (C2:0) (0, 250, 500, 750, 1500, 2500, and 5000 pmoles) in the dark. Samples were illuminated with UL light to generate the cross-links and resolved on SDS gels and prepared as described above. For preparative work, the reactions were scaled up 10–20-fold maintaining a FACS: \( ^{3H} \)APNA molar ratio of 1:3 as under these conditions, there was maximal labeling of the enzyme. We estimated the efficiency of labeling purified FACS with \( ^{3H} \)APNA to be between 25 and 30%.

**Purification of the \( ^{3H} \)APNA-labeled Peptide and Protein Sequencing—** Following UV illumination and confirmation that the enzyme was labeled with \( ^{3H} \)APNA, the samples were digested with trypsin (100 \( \mu \)g/mL) at 37 °C for 1 h at pH 8. For the experiment using purified enzyme or \( ^{3H} \)APNA alone. The resultant peptides were resolved on a Nucleosil column (250 \( \times \) 4.6 mm, C18, Phenomenex) and developed with a linear gradient of acetonitrile with 0.1% trifluoroacetic acid (0–100% in 60 min). Peptides were detected at 230 nm using a Beckman System Gold High Pressure Liquid Chromatography System. One-milliliter fractions were collected and aliquots assayed for radioactivity. Peptides containing a radioactive signal were identified and repurified by a second round of HPLC. Following purification, peptides were subjected to NH\(_2\)-terminus peptide sequence analysis. Peptide sequencing was done at the Amino Acid Analysis and Peptide Sequencing Core Facility of the Wadsworth Center of the New York State Department of Public Health using an Applied Biosystems 477A Protein Sequencer.

**Structural Predictions and Amino Acid Sequence Comparisons—** The predicted structure for the \( E. coli \) FACS, generated using SWISS-MODEL and visualized using SWISS-Pdb Viewer has been previously described (15). Figures of the predicted structure of the \( E. coli \) FACS were downloaded using MolScript v.2.2.1 on a Silicon Graphics Workstation. Protein sequence comparisons were performed using BioSCAN (31) or MultAlign v.5.3.3 (32), with sequences that are conserved among the fatty acyl-CoA synthetases or members of the adenylate forming enzyme or \( ^{3H} \)APNA alone. The resultant peptides were resolved on a Nucleosil column (250 \( \times \) 4.6 mm, C18, Phenomenex) and developed with a linear gradient of acetonitrile with 0.1% trifluoroacetic acid (0–100% in 60 min). Peptides were detected at 230 nm using a Beckman System Gold High Pressure Liquid Chromatography System. One-milliliter fractions were collected and aliquots assayed for radioactivity. Peptides containing a radioactive signal were identified and repurified by a second round of HPLC. Following purification, peptides were subjected to NH\(_2\)-terminal peptide sequence analysis. Peptide sequencing was done at the Amino Acid Analysis and Peptide Sequencing Core Facility of the Wadsworth Center of the New York State Department of Public Health using an Applied Biosystems 477A Protein Sequencer.

**RESULTS**

**Purification and Kinetic Properties of FACS—** Our previous work identified a conserved 25-amino acid residue segment within FACS, which we hypothesized is part of the fatty acid-binding domain within the enzyme (27). In order to test this hypothesis, the enzyme containing an amino-terminal hexameric histidine tag (His\(_6\)-FACS) was purified using Ni\(^2+\) chelation chromatography and kinetically characterized in the presence of 0.1% Triton X-100 prior to photolabeling experiments using an affinity labeled long-chain fatty acid (Fig. 1). The enzyme was not active in the absence of added nonionic detergent or phospholipid indicating that catalysis is likely to occur
at the membrane. The His-tagged enzyme displayed classical Michaelis-Menten kinetics at oleate concentrations between 0 and 5 μM, which allowed apparent kinetic parameters to be defined. The apparent $V_{\text{max}}$ and $K_m$ for purified His$_6$-FACS were 77.7 nmol/min/mg protein and 1.1 μM oleate, respectively. These values were consistent with published values for the purified native enzyme from E. coli (25).

Circular Dichroism Spectra of Purified FACS—We have recently proposed a three-dimensional model for FACS developed using SWISS-MODEL and visualized using Swiss-Pdb Viewer (15). This prediction suggests FACS adopts a three-dimensional architecture similar to that defined for firefly luciferase and phenylalanine activating subunit (PheA) of gramicidin synthetase 1 (33, 34). The crystal structures for these adenylate-forming enzymes have been solved and demonstrate that both contain a large NH$_2$-terminal domain and small COOH-terminal domain separated flexible linker that results in a cleft. Specific amino acid residues exposed within this cleft as well as those in the flexible linker are hypothesized to represent the catalytic core of FACS. For firefly luciferase, the NH$_2$-terminal domain consists of a β-barrel and several β-strands. These β-strands are flanked by α helices to form an α-β-α 5-layered structure. (33). The far UV circular dichroism spectrum of purified FACS was defined in order to assess the α and β secondary structure. The far UV (240–178 nm) CD spectra for the purified enzyme was determined as detailed under “Experimental Procedures” and analyzed using SELCON (Fig. 2). These data indicated this enzyme contained 47% α, 17% β, 13% β turn, and 23% other structure. The relative amounts of α and β structure were consistent with predicted the three-dimensional model for the E. coli FACS (15) and similar to those for firefly luciferase and phenylalanine activating subunit (PheA) of gramicidin synthetase 1 (33, 34).

Labeling Purified FACS with [3H]APNA—APNA is similar in length to palmitate (C16:0), yet is different by containing an azidophenoxyl group attached to nonanoic acid via an ether linkage. DiRusso et al. (18) have used the CoA derivative of APNA to localize the fatty acyl-CoA-binding domain of the transcription factor FadR. These investigators were able to demonstrate the APNA-CoA covalently modified a region within the carboxyl-terminal domain of the protein previously predicted to bind ligand from studies using site-directed mutagenesis (16, 35). Aliquots of purified FACS (400 pmol) were incubated with increasing concentrations of [3H]APNA in the dark as detailed under “Experimental Procedures.” Following illuminations with UV light, individual samples were resolved on 12% SDS-polyacrylamide gels and subjected to fluorography. As shown in Fig. 3A, [3H]APNA labeling to purified FACS increased with increasing concentration of ligand. Labeling of the enzyme was close to saturation between 1500 and 2000 pmol of APNA (and estimated to represent a 25–30% efficiency of labeling) (Fig. 3B). In order to establish the specificity of [3H]APNA labeling, purified enzyme (400 pmol) was incubated with 1500 pmol of [3H]APNA and increasing concentrations of unlabeled palmitate (250–2500 pmol) in the dark prior to cross-linking. Following cross-linking and SDS-polyacrylamide gel electrophoresis, the gels were subjected to fluorography and analyzed. The labeling of FACS with [3H]APNA was effectively eliminated using increasing concentrations of palmitate (Fig. 4A). As illustrated in Fig. 4B, a 50% reduction of [3H]APNA labeling was achieved using 1200–1400 pmol of palmitate. These data indicated that labeling of the enzyme by [3H]APNA was, (1) saturable and (2) specific on the basis of competition by palmitate. The specificity of APNA labeling was further investigated by incubating the purified enzyme (400 pmol) with increasing concentrations of fatty acids with differing chain lengths (C24:0, C10:0, C6:0, and C2:0) (Fig. 5). Palmitate was the most effective fatty acid in competing out [3H]APNA binding followed by decanoate and hexanoate. Lignocerate and acetate were unable compete for [3H]APNA binding. These data argue that the site within the enzyme modified by [3H]APNA is specific for long-chain fatty acids, but contains some specificity toward medium-chain fatty acids. These results are consistent with earlier kinetic studies of the enzyme (25). These findings demonstrated that [3H]APNA could be used as an effective affinity label to probe that fatty acid-binding domain of FACS.

Identification of a [3H]APNA-labeled Tryptic Peptide of FACS—In order to define the region of FACS that had become modified, the experiments were scaled up 10–20-fold and following UV illumination, the [3H]APNA-labeled FACS was proteolyzed with trypsin as detailed under “Experimental Procedures.” As noted above, the specific labeling of FACS with APNA was estimated to be between 25 and 30%. As a control, FACS alone was proteolyzed under identical conditions. The proteolyzed samples were resolved on a Nucleosil column and developed using a linear acetonitrile gradient (0–100% acetonitrile containing 0.1% trifluoroacetic acid). Peptides were detected at 230 nm and 1.0-ml fractions collected. Aliquots from

3 J. D. Weimar and P. N. Black, unpublished observations.
each fraction were counted to detect the [3H]APNA-labeled peptide(s). [3H]APNA alone was also resolved by reverse-phase HPLC under the same conditions. Fig. 6 shows a representative experiment using this approach and the identification of a single labeled peptide, T33, with an increased retention time on the reverse phase column when compared with the unlabeled and proteolyzed FACS. The T33 sample contained the majority of the protein-bound [3H]APNA (70–75%), while the remaining [3H]APNA was distributed among other peptide fractions. There was consistent labeling of T33 with [3H]APNA in four independent experiments. [3H]APNA was resolved in the absence of protein to 1) define the retention characteristics of this compound, 2) assess purity prior to photolabeling, and 3) define if there were fragmentation products. As shown in Fig. 6, APNA alone had an average retention time between 15 and 17 min on the Nucleosil column. The [3H]APNA-labeled peptide (T33) was isolated and purified using reverse-phase HPLC and sequenced using automated Edman degradation. The NH2-terminal sequence of this peptide, PDATDEIIK, corresponds to residues 422–430 in FACS and is adjacent to the region of the enzyme that contains the FACS signature motif (residues 431–455) (Fig. 7). The APNA-labeled peptide, T33, is therefore likely to contain part or the entire region of the enzyme that includes the FACS signature motif. These data fully support our previous observations using alanine-scanning mutagenesis of the fadD gene that this region of the enzyme is involved in fatty acid binding (27).

Interpretation of the Fatty Acid-binding Site within the Predicted Structure for FACS—The E. coli FACS is a member of the AMP-binding protein superfamily and contains signature sequences predicted to specify ATP binding (ATP/AMP signature motif) (36–39). Using the crystallographic information for two enzymes containing the ATP/AMP signature motif (firefly luciferase (33) and the phenylalanine activating subunit (PheA) of gramicidin synthetase 1 (34)), we proposed a three-dimensional model for the E. coli FACS (Fig. 8A (15)). This model predicted the region we identified as the FACS signature (hypothesized to specify fatty acid binding) formed a β, β-turn-β structure, which was on the same face of the enzyme as elements that comprise the ATP/AMP signature motif (boxed in Fig. 8A). The identification of an APNA-labeled peptide, beginning with Pro422, adjacent to and contiguous with the FACS signature (involved
in fatty acid binding) confirmed our hypothesis regarding the fatty acid-binding domain making this model quite compelling. On the basis of the predicted structure of this enzyme, the region bound by $\beta_1$ and $\beta_2$ of the FACS signature motif (Fig. 7) contributes to a cavity that we predict is the fatty acid-binding site. Fig. 8B illustrates the $\alpha$-carbon trace of the FACS signature motif (Asn 431-Lys455) in the same orientation as shown in Fig. 8A, which emphasizes the $\beta$, $\beta$-turn-$\beta$ structure. On the basis of this information, it seems likely that the region of FACS, which includes the cleft separating the two domains of the enzyme, represents the catalytic core of the enzyme. We suspect that upon ligand binding (ATP and fatty acid), the two domains close to facilitate the formation of the fatty acyl adenylate.

**DISCUSSION**

Fatty acyl-CoA synthetase represents a pivotal enzyme in lipid metabolism. The goal of our work is to define the biochemical mechanisms that underpin the role of FACS in the transport of exogenous fatty acids. We hypothesize fatty acid transport proceeds across the cell membrane through the vectorial esterification of exogenous fatty acids. In E. coli, the esterification of exogenous long-chain fatty acids with coenzyme A is tightly coupled to a regulatory network linking fatty acid import to the differential expression of genes involved in fatty acid degradation and biosynthesis (15, 20, 21). There is emerging evidence that in a number of eukaryotic systems, the fatty acid activation-coupled transport also occurs, indicating this is a

**FIG. 6.** Reverse phase HPLC profile of tryptic peptides generated from FACS alone (gray trace) or FACS cross-linked with $[^{3}H]$APNA (black trace). The bar graph indicates the dpm of $[^{3}H]$APNA alone (open bars) or FACS-$[^{3}H]$APNA tryptic peptides (closed bars). The APNA-labeled peptide, T33 (boxed), was purified using a second round of HPLC and identified by NH$_2$-terminal amino acid sequencing.

**FIG. 7.** A, FACS signature (wavy line) and flanking region sequenced as the tryptic peptide (double underline) of the E. coli enzyme. B, alignments of bacterial, yeast, and mammalian fatty acyl-CoA synthetases and firefly luciferases corresponding to the region of the bacterial enzyme containing the FACS signature and labeled with $[^{3}H]$APNA. Only the differences between the sequences are given. The numbers flanking each sequence specify amino acid residue number for corresponding regions containing the FACS signature motif. Ec (Escherichia coli), Hi (hemophilus influenzae), Bs (Bacillus subtilis), Mt (Mycobacterium tuberculosis), Po (Pseudomonas oleovarans). The accession numbers are shown on the left.

**FIG. 8.** A, model of the E. coli fatty acyl-CoA synthetase developed using the Swiss-Model Protein Modeling Server and visualized using MolScript. The predicted structure begins with residue Thr$^{313}$ of the native enzyme. Residues that comprise the peptide modified with APNA and continuing into the FACS signature motif are in shown in green (Pro$^{325}$-Lys$^{405}$). $\alpha$ denotes the predicted a helix upstream from the FACS signature motif; $\beta_1$, $\beta_2$, and $\beta_3$ denote the $\beta$, $\beta$-turn-$\beta$ structure of the FACS signature; and L denotes the linker between the large NH$_2$-terminal and small COOH-terminal domains of the enzyme. The boxed region denotes the $\beta$-loop-$\beta$ structure that comprises the first sequence element of the ATP/AMP signature motif while the residue identified with an asterisk is Glu$^{361}$ (within the second sequence element of the ATP/AMP signature motif), which is conserved in all adenylation-forming enzymes. B, $\alpha$-carbon tracing of the FACS signature motif (Asn$^{431}$-Lys$^{455}$) highlighting the $\beta$, $\beta$-turn-$\beta$ structure that is proposed to contribute to the fatty acid binding pocket within the enzyme.
common mechanism promoting the movement of exogenous fatty acids across the membrane in a highly regulated manner (24, 39–40). In the present report, we provide experimental evidence defining the regions within FACS, which are essential for activity and estimate structural characteristics of the protein.

The present work represents the first direct biochemical investigation into the identity and location of the fatty acid-binding domain within FACS. We have demonstrated the affinity labeled long-chain fatty acid, APNA, precisely modifies a region of the enzyme, which includes the FACS signature motif. This work confirms our hypothesis, based on alanine-scanning mutagenesis of the FACS structural gene (fadD), that this region of the enzyme contributes to the fatty acid-binding site (27). From these data and previous analyses of fadD alleles with single amino acid substitutions, a number of conclusions can be drawn regarding the ligand-binding sites within the enzyme. First, the fatty acid-binding site, which includes the fatty acid-binding site extends into the cleft where we presume the fatty acyl-CoA synthetase is, likewise, involved in fatty acid import (39–40). In the present report, we provide experimental evidence defining the regions within FACS, which are essential for the second carboxyl-containing substrate is described by specific residues corresponding to the region that in specificity for the second carboxyl-containing substrate is described by specific residues corresponding to the region that in ATP binding) are in juxtaposition with those that comprise the ATP/AMP signature motif (hypothesized to be involved in ATP binding) are in juxtaposition with those that comprise the ATP/AMP signature motif (hypothesized to be involved in ATP binding). The first element of the ATP/AMP signature motif involved in fatty acid import and activation of exogenous long-chain fatty acids (23). An additional protein within yeast, Fat1p, is involved in fatty acid import and has an acyl-CoA synthetase activity, suggesting it may work in conjunction with either Faa1p or Faa4p to promote coupled fatty acid import activation (24, 44). In mammalian cells, the emerging evidence suggests that fatty acyl-CoA synthetase is, likewise, involved in fatty acid import (39–41). The challenges that we are presently faced with not only include detailed mechanistic and structural investigations on the FACS families of enzymes, but also to more precisely determine how these enzymes function in fatty acid import. Our laboratory is presently investigating these questions.

Acknowledgments—Thanks are due to Kristin Fox (Union College, Schenectady, New York) for assistance with MolScript. The Amino Acid Analyses and Peptide Sequencing Core Facility of the Wadsworth Center of the New York State Department of Public Health directed by Frank Maley and Li-Ming Chanchein provided peptide sequencing.

REFERENCES

1. Carmen, G. M., and Zeitmiet, G. M. (1996) J. Biol. Chem. 271, 13293–13296
2. Hillgartert, F. B., Salaiti, L. M., and Goodridge, A. G. (1995) Physiol. Rev. 75, 47–76
3. Glick, B. S., and Rothman, J. E. (1987) Nature 326, 309–312
4. Gordon, J. J., Duronio, R. S., Rudnick, D. A., Adams, S. P., and Gokel, G. W. (1991) J. Biol. Chem. 266, 8647–8650
5. McLaughlin, S., and Aderem, A. (1990) Trends Biochem. Sci. 20, 270–276
6. Nimchuk, Z., Marous, E., Kjemstrup, S., Leister, R. T., Katagari, F., and Dangl, J. L. (2000) Cell 101, 353–363
7. Fujimoto, Y., Tsunomori, M., Sumiya, T., Nishida, H., Sakuma, S., and Fujita, K. (1985) Prostaglandins Leukotriens Essent. Fatty Acids 25, 255–258
8. Fulcheri, R., Gamberucci, A., Scott, H. M., Giunti, R., Burchell, A., and Benedetti, A. (1995) Biochem. J. 307, 391–397
9. Lai, J. C., Liang, B. B., Jarri, E. J., Cooper, A. J. L., and Lu, D. R. (1993) Res. Commun. Chem. Pathol. Pharmacol. 82, 331–338
10. Yamashita, A., Watanabe, M., Tonegawa, T., Sugita, T., and Waku, K. (1995) Biochem. J. 312, 301–308
11. Aihund, G. P., Aburnrad, N. A., Amzi, E. Z., and Grimoldi, P. A. (1994) in Fatty Acids and Lipids: Biological Aspects (Simpouos, G. C., and Tremol, E., eds) Karger Press, Basel, Switzerland
12. Shrago, E., Waldgriegi, G., Husko, A., and Di Russo, C. C. (1995) Prostaglandins Leukotriens Essent. Fatty Acids 52, 163–166
13. Rich, G. T., Comerford, J. G., Graham, S., and Dawson, A. P. (1995) Biochem. J. 306, 705–708
14. Færgeman, N. J., Ballegaard T., Knudsen, J., Black, P. N., and DiRusso, C. C. (2000) Subcell. Biochem. 34, 175–231
15. Di Russo, C. C., Black, P. N., and Weimar, J. D. (1999) Prog. Lipid Res. 38, 129–197
16. Raman, N., Black, P. N., and Di Russo, C. C. (1997) J. Biol. Chem. 272, 30645–30650
17. Di Russo, C. C., Tsvetnitsky, V., Hajrup, P., and Knudsen, J. (1998) J. Biol. Chem. 273, 33652–33659
18. Di Russo, C. C., Heimert, T. L., and Metzger, A. K. (1992) J. Biol. Chem. 267, 8605–8609
19. Hertz, R., Magenheim, J., Berman, I., and Bar-Tana, J. (1998) Nature 392, 512–516
20. Di Russo, C. C., Black, P. N. (1999) Mol. Cell. Biochem. 192, 41–52
21. Black, P. N., Færgeman, N. J., and Di Russo, C. C. (2000) J. Nutr. 130, 305–309
22. Overath, P., Pauli, G., and Schairer, H. U. (1989) Eur. J. Biochem. 7, 559–574
23. Knoll, L. J., Johnson, D. R., and Gordon, J. L. (1995) J. Biol. Chem. 270, 10861–10867
24. DiRusso, C. C., Connell, E., Færgeman, N. J., Hansen, J.-K., Knudsen, J., and Black, P. N. (2000) *Eur. J. Biochem.* **267**, 4422–4433
25. Kameda, K., and Nunn, W. D. (1981) *J. Biol. Chem.* **256**, 5702–5707
26. Black, P. N., DiRusso, C. C., Metzger, A. K., and Heimert, T. L. (1992) *J. Biol. Chem.* **267**, 25513–25520
27. Black, P. N., Zhang, Q., Weimar, J., and DiRusso, C. C. (1997) *J. Biol. Chem.* **272**, 4896–4903
28. DiRusso, C. C., Metzger, A. K., and Heimert, T. L. (1992) *J. Biol. Chem.* **267**, 25513–25520
29. Sreerama, N., and Woody, R. W. (1993) *Anal. Biochem.* **209**, 32–44
30. Laemmli, U. K. (1970) *Nature* **227**, 680–685
31. Singh, R. K., Hoffman, D. L., Tell, S. G., and White, C. T. (1996) *Comput. Appl. Biosci.* **12**, 191–196
32. Corpet, F. (1988) *Nucleic Acids Res.* **16**, 10881–10889
33. Conti, E., Franks, N. P., and Brick, P. (1996) *Structure* **4**, 287–298
34. Conti, E., Stachelhaus, T., Marahiel, M. A., and Brick, P. (1997) *EMBO J.* **16**, 4174–4183
35. Raman, N., and DiRusso, C. C. (1995) *J. Biol. Chem.* **270**, 1092–1097
36. Babbitt, P. C., Kenyon, G. L., Martin, B. M., Charest, H., Slyvestre, M., Scholten, J. D., Chang, K.-H., Liang, P. H., and Dunaway-Mariano, D. (1992) *Biochemistry* **31**, 5594–5604
37. Pavela-Vranic, M., Pfeifer, E., van Liempt, H., Schafer, H.-J., von Dohren, H., and Kleinkauf, H. (1994) *Biochemistry* **33**, 6276–6283
38. Chang, K. H., Xiang, H., and Dunaway-Mariano, D. (1997) *Biochemistry* **36**, 15650–15659
39. Gargiulo, C. E., Stuhl-Kouper, S. M., and Schaffer, J. E. (1999) *J. Lipid Res.* **40**, 881–892
40. Coe, N. B., Smith, A. J., Frohnert, B. I., Watkins, P. A., and Bernlohr, D. A. (1999) *J. Biol. Chem.* **274**, 36300–36304
41. Schaffer, J. E., and Lodish, H. F. (1994) *Cell* **79**, 427–436
42. van Beilen, J. B., Eggink, G., Enequist, H., Bos, R., and Witholt, B. (1992) *Mod Microbiol.* **6**, 3121–3136
43. Knoll, L. J., Johnson, D. R., and Gordon, J. I., (1994) *J. Biol. Chem.* **269**, 16348–16356
44. Færgeman, N. J., DiRusso, C. C., Elberger, A., Knudsen, J., and Black, P. N. (1997) *J. Biol. Chem.* **272**, 8531–8538
Affinity Labeling Fatty Acyl-CoA Synthetase with 9-p-Azidophenoxy Nonanoic Acid and the Identification of the Fatty Acid-binding Site
Paul N. Black, Concetta C. DiRusso, David Sherin, Robert MacColl, Jens Knudsen and James D. Weimar

*J. Biol. Chem.* 2000, 275:38547-38553.
doi: 10.1074/jbc.M006413200 originally published online September 19, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M006413200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 43 references, 19 of which can be accessed free at http://www.jbc.org/content/275/49/38547.full.html#ref-list-1