Isolation of a Functional Transferase Component from the Rat Fatty Acid Synthase by Limited Trypsinization of the Subunit Monomer

FORMATION OF A STABLE FUNCTIONAL COMPLEX BETWEEN TRANSFERASE AND ACYL CARRIER PROTEIN DOMAINS*

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Limited trypsinization of rat fatty acid synthase monomers results in cleavage at sites protected in the native dimer. A 47,000-Da polypeptide containing the transferase component was isolated from the digest and its location in the multifunctional polypeptide established. Both acetyl and malonyl moieties are transferred stoichiometrically from CoA ester to this polypeptide and each can replace the other, confirming that the 8,000-Da acyl carrier protein domain. The transferase domain could be isolated in association with the 8,000-Da acyl carrier protein domain of the multifunctional fatty acid synthase by limited trypsinization of the subunit monomer. The transferase was also able to utilize as acyl acceptor the Escherichia coli acyl carrier protein and the acyl carrier protein domain of the multifunctional fatty acid synthase. When the fatty acid synthase monomer was trypsinized under milder conditions, the 47,000-Da transferase domain could be isolated in association with the 8,000-Da acyl carrier protein domain. The transferase is capable of translocating substrate moieties from CoA ester donors to the associated acyl carrier protein. The results provide the first direct evidence that, in the head-to-tail oriented fatty acid synthase homodimer, functional communication between the transferase domain located near the end of one polypeptide and the acyl carrier protein domain located at the opposite end of the other polypeptide is facilitated by a stable physical interaction between these domains.

The animal fatty acid synthase is a multifunctional protein consisting of two identical head-to-tail oriented subunits, each carrying seven functional domains (2). Some of these domains have been dissected from the homodimer as independent functional proteins by limited proteolysis, supporting the theory that the multifunctional protein may have evolved by the fusion of genes encoding discrete monofunctional enzymes.

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EXPERIMENTAL PROCEDURES

Reagents and Enzymes—Radiolabeled acetyl-CoA and malonyl-CoA were obtained from Amersham Corp.; unlabeled acyl-CoAs were obtained from PL Biochemicals or Sigma. Pantetheine (Sigma) was reduced to the free thiol form with 2% sodium amalgam (6). ATP:citrate lyase was purified from rat liver (7), and malic dehydrogenase was obtained from Sigma. Escherichia coli ACP was obtained from Dr. John E. Cronan, Jr. (University of Illinois, Urbana, IL). All enzyme units are defined as the amount of enzyme required to utilize 1 μmol of substrate/min.

Isolation of the Transferase Domain—Fatty acid synthase from rat liver was purified to near homogeneity (8). Thioesterase domains were removed by limited trypsinization of the native dimer, and the remaining nicked-core dimers were purified by gel filtration and ammonium sulfate precipitation (9). The nicked-core dimers were dissociated into nicked-core monomers by incubation in 100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM dithiothreitol, at 4 °C, typically for about 7 days. The nicked-core monomers were then digested with trypsin (trypsin:synthase, 150, w/w) at 10 °C, and the reaction was stopped, typically after 5–6 h, by the addition of trypsin inhibitor (trypsin:inhibitor, 1:5); in later experiments, when it had been established that phenylmethylsulfonfyl fluoride did not inhibit transferase activity, this reagent (0.5 mM) was used in place of trypsin inhibitor. In some experiments the trypsin:asynthase ratio was decreased to 1:75, and the incubation time was shortened to 4 h at 10 °C; in these experiments the buffer used was 50 mM potassium phosphate (pH 7.0), 1 mM EDTA. Progress of the dissociation of nicked-core dimers into nicked-core monomers and of the subsequent digestion with trypsin was monitored by rapid high performance liquid chromatography on a Zorbax GF-250 column (Du Pont) equilibrated with 150 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA. Fractionation of the bulk digest was achieved on a gel filtration column of Ultragel AcA54 with the same eluant. Proteins in the eluant were analyzed by SDS-polyacrylamide gel electrophoresis (10), and fractions containing the 47-kDa species were pooled.

Assay of Transferase Activity—Transferase activity was assayed using acyl-CoA thioesters as substrates and pantetheine as a model acceptor. The free CoA produced by the transferase was subsequently assayed spectrophotometrically in a coupled reaction between ATP:citrate lyase and malate dehydrogenase. Initially, reaction mixtures contained 100 mM potassium phosphate buffer (pH 7.0), acyl-CoA, 1.7 mM pantetheine, and 0.2–4 μM of the isolated transferase in a final volume of 25 μl. The incubation period was 10 min at 20 °C. A mixture containing (concentrations in a final volume of 0.2 ml) 250 mM Tris-HCl (pH 7.5), 25 mM MgCl2, 7.5 mM ATP, 5 mM dithiothreitol, 0.3 mM NADH, 0.4 unit of rat liver ATP-citrate lyase, and 2

1 The abbreviations used are: ACP, acyl carrier protein; SDS, sodium dodecyl sulfate; HPLC, high performance liquid chromatography.

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units of malate dehydrogenase was then added. The ATP-citrate lyase reaction, at 30 °C, was started by the addition of potassium citrate (50 mM, final concentration), and the total amount of NADH oxidized was measured at 340 nm 1 min later. The utility of the assay depends on the low Km and high vmax of the ATP:citrate lyase for its CoA substrate (11) permitting rapid scavenging of even trace amounts of CoA. Control experiments with known amounts of CoA (1-12 nmol) indicated that stoichiometric utilization of CoA and oxidation of NADH was achieved in less than 30 s. Under the conditions used chemical hydrolysis of the acyl-CoA was negligible. Transferase activity with E. coli ACP as acceptor was assessed radiochemically, using [2-14C]malonyl-CoA; the components of the coupled citrate lyase/malate dehydrogenase reactions were omitted. Reactions were continued for 10 min at 20 °C and stopped by the addition of 1.5 ml of 10% trichloroacetic acid. Bovine serum albumin (25 μl of 5 mg/ml) was added as a coprecipitant, samples were left at 0 °C for 1 h, and the radiolabel was collected and washed on Millipore filters (HA 0.45 μm), dried, and assayed for radioactivity.

Amino Acid Sequencing—The fragment containing the transferase component was separated from the tryptic digest by SDS-polyacrylamide gel electrophoresis (10), blotted onto a polyvinylidene difluoride membrane (12), and subjected to Edman degradation on an Applied Biosystems 477A Sequencer.

Translocation of [14C] Malonyl Moieties to the Fatty Acid Synthase—Transferase (6.1 μM) in 100 mM phosphate buffer (pH 7.5), 1 mM EDTA was incubated with 5 mM iodoacetamide for 30 min at room temperature. Excess reagent was removed by extensive dialysis against 100 mM phosphate buffer (pH 7.0), 1 mM EDTA. Then the iodoacetamide-treated transferase (4.5 μM), in 100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, was incubated with 40 μM [2-14C]malonyl-CoA at room temperature for 10 min. Excess radiolabeled malonyl-CoA was removed by repeated passage of the mixture through Bio-Gel P-6DG spin columns. Removal of free radiolabeled transferase was confirmed by quantitative precipitation of a portion of the radiolabeled protein with trichloroacetic acid. The radiolabeled transferase (0.57 μM) in 100 mM potassium phosphate (pH 7.0), 1 mM dithiothreitol, 1 mM EDTA) was incubated with fatty acid synthase, monomer or dimer (1.4 μM), at 4 °C for 10 min, and the mixture was then fractionated at 4 °C on a Zorax GF-250 HPLC column eluted with 100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA. Under these conditions no change in the monomer/dimer equilibrium occurred. The nature of the radiolabeled ester linkage to the fatty acid synthase, i.e. thioester or oxygen ester, was determined by assessing lability to hydroxylamine treatment. Thus the radiolabeled protein was treated with neutralized hydroxylamine (0.2 M, final concentration) at 4 °C for 40 min, and residual protein-bound radioactivity was determined following precipitation with trichloroacetic acid.

RESULTS AND DISCUSSION

Isolation of the Transferase Component—Previous attempts to dissect out functional domains of the mammalian fatty acid synthase by limited proteolysis met with limited success, since much of the enzyme core appeared to be resistant to proteases (13-15). These resistant core polypeptides consisted primarily of two long fragments derived from the amino-terminal (125 kDa) and carboxyl-terminal (95 kDa) halves of the protein and a small fragment (12 kDa) that originated from the central region of the subunits (16). Pairs of these 125 + 95 + 12 kDa core polypeptides remained associated as “nicked-core dimers” (13, 15, 16). Recently, we found that cystic sites of proteolytic attack could be exposed by cold-induced dissociation of the nicked-core dimers into nicked-core monomers; these nicked-core monomers were subjected to further treatment with trypsin, and the previously resistant 125- and 95-kDa core polypeptides were found to be extensively degraded; analysis of the digest by SDS-polycrylamide gel electrophoresis revealed three relatively stable polypeptide species of 47, 30, and 12 kDa (16). The 47-kDa polypeptides were isolated from the digest by gel filtration, incubated with radiolabeled malonyl-CoA, and electrophoresed on a SDS-polycrylamide gel; the radiolabeled malonyl moieties were found associated with the 47-kDa polypeptide (Fig. 1), suggesting that this polypeptide fragment contains the transferase activity of the fatty acid synthase. Amino-terminal sequencing established that the first residue corresponded to Gly385 of the parent multifunctional polypeptide (17). Although the carboxyl terminus of the 47-kDa fragment was not determined directly, a likely candidate would be Arg385, which would generate a fragment of predicted molecular mass 47,766 Da. This fragment contains a sequence Gly-His-Ser-Leu-Gly which is identical to that of short radiolabeled peptides isolated from digests of mammalian fatty acid synthases that had been labeled with either acetyl- or malonyl-CoA (18, 19). The serine residue, which we now know is located at residue 581 of the parent polyfunctional polypeptide (17), had been implicated as the active site residue of the transferase domain (18).

Formation of an Acyl-enzyme Intermediate—To confirm that the 47-kDa fragment possessed properties consistent with a transferase function, the polypeptide was incubated with radiolabeled malonyl- and acetyl-CoA, and binding to the protein was assayed (Table I). Approximately 1 mol of substrate was bound per mol of 47-kDa polypeptide (first incubations, Table I). Furthermore, loading of both substrate moieties was reversible, and each substrate competed for the binding site of the other (second incubations, Table I). This observation is consistent with the hypothesis, advanced (20) and confirmed (18, 19) earlier, that the fatty acid synthase loads its substrates via a single shared site rather than two separate sites and that the loading is random rather than sequential.

Substrate Specificity and Kinetics—The kinetics and substrate specificity of the isolated transferase were determined with pantotheine as a model acyl acceptor in a new assay system. The assay procedures used previously required chromatographic separation of radiolabeled substrates and products, whereas the new assay involves only the spectrophotometric measurement of free CoA produced in the initial trans-
Isolation of the Fatty Acid Synthase Transferase Domain

Stoichiometry of acetyl and malonyl binding to the isolated transferase and replacement of one substrate by the other

The 47-kDa polypeptide (1.1 μM) was first incubated with either [2-14C]malonyl-CoA (36 μM, 55 Ci/mol) or [1-14C]acyetyl-CoA (40 μM, 6.5 Ci/mol) for 2 min at 10 °C. Reactions were either terminated by the addition of perchloric acid (10%, final concentration) or unlabeled substrate (50 μM) was added and a second incubation performed for 2 min at 10 °C. Carrier protein (1 mg/ml bovine serum albumin) was added, proteins were collected by centrifugation, washed with 5% perchloric acid, and radioactivity assayed.

<table>
<thead>
<tr>
<th>First incubation, labeled substrate</th>
<th>Second incubation, unlabeled substrate</th>
<th>Labeled substrate bound to 47-kDa polypeptide</th>
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<tbody>
<tr>
<td>[2-14C]Malonyl-CoA</td>
<td>0.72 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>[2-14C]Malonyl-CoA Acetyl-CoA</td>
<td>0.28 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>[1-14C]Acetyl-CoA</td>
<td>0.50 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>[1-14C]Acetyl-CoA Malonyl-CoA</td>
<td>0.27 ± 0.01</td>
<td></td>
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</tbody>
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Kinetics and substrate specificity of the isolated 47-kDa transferase
Transferase activity of the 47-kDa fragment (0.17–3.4 μM) was assessed with various acyl-CoA esters as acyl donors and either pantetheine (1.6 mM) as acceptor, using the coupled ATP-citrate lyase/malate dehydrogenase assay or E. coli ACP (23.1 μM) as acceptor, using the radiochemical assay. The values for the apparent Michaelis-Menten constants, K+', and the apparent maximum velocities, Vmax', calculated from double-reciprocal plots, are presented with standard errors of their means. Additional studies (details not shown) were carried out with various potential acyl donors; specific activities of 0.042 and 0.285 units/mg were observed at optimal concentrations of octanoyl-CoA and phenylacetyl-CoA, respectively, and no activity was detectable with decanoyl-CoA as acyl donor.

<table>
<thead>
<tr>
<th>Acceptors</th>
<th>ACP</th>
<th>Pantetheine</th>
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<tbody>
<tr>
<td>Malonyl-CoA</td>
<td>22 ± 0</td>
<td>0.30 ± 0</td>
</tr>
<tr>
<td>Acetyl-CoA</td>
<td>58 ± 1</td>
<td>0.31 ± 0</td>
</tr>
<tr>
<td>Butyryl-CoA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Hexanoyl-CoA</td>
<td>ND</td>
<td>ND</td>
</tr>
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* Correlation coefficients for the linear double reciprocal plots ranged from 0.967 to 0.985.

** ND, not determined.

Activity with E. coli ACP as the acceptor was assayed radiochemically; no labeling of the ACP was observed in the absence of transferase. The isolated transferase showed high specific activity for the transfer of acetyl and malonyl moieties from CoA to pantetheine thiols. Enzyme that had been heated from CoA to pantetheine thiols. Enzyme that had been heated for 10 min at 65 °C was completely inactive. Activity decreased with increasing acyl chain length and was undetectable for substrates with more than eight carbon atoms in the acyl chain (Table II). This specificity is similar to that observed when the ability of acyl-CoAs to prime fatty acid synthesis was compared (21, 22). However transferase activity of the the 47-kDa fragment with acetyl-CoA as donor is approximately 5 times that with butyryl-CoA and 22 times that with phenylacetyl-CoA, whereas in the intact multifunctional complex acetyl- and butyryl-CoA support similar rates of fatty acid synthesis, about 6 times that supported by phenylacetyl-CoA. The most likely explanation is that the rate-limiting step in the overall reaction is not the transfer of substrates to and from the enzyme (20) but is probably the first condensation or ketoreduction reaction (23).

The K+ values for the various acyl donors reported in Table II were determined at the same single concentration of pantetheine. However the actual values are dependent on the concentration of the pantetheine acceptor used and therefore are reported as apparent values, K+'. Thus when the dependence of activity on malonyl-CoA concentration was assessed at various concentrations of pantetheine acceptor, or the dependence on pantetheine concentration assessed at various malonyl-CoA concentrations, double-reciprocal plots yielded a series of parallel lines (Fig. 2, A and B). Secondary double-reciprocal plots of the K+ values for one substrate, obtained at various fixed concentrations of the other substrate, against the reciprocal of the concentration of the other substrate gave straight lines (Fig. 2C). From the intercepts on the y axis the K+ value for malonyl-CoA at infinite pantetheine concentration (K+∞) was determined as 1.36 mM and the K+∞ for pantetheine was calculated to be 0.82 mM. These values are similar to those calculated for the monofunctional malonyl transferases isolated from plants (24) and bacteria (25). The ability of the transferase to form an acyl-enzyme complex in the absence of acceptor (Fig. 1 and Table I) is also consistent with such a mechanism. Although these kinetic studies are useful for assessing the substrate specificity of the transferase and for comparison of its possible mechanism with that of the naturally occurring multifunctional transferases found in plants and microorganisms, the magnitude of the kinetic parameters themselves may be of little physiological significance. In the native multifunctional fatty acid

![Fig. 2. Concentration dependence of the K+ of the 47-kDa transferase for donor and acceptor.](image-url)

A double-reciprocal plots of transferase activity at various concentrations of malonyl-CoA and different fixed concentrations of pantetheine: 0.6 mM (○); 1.2 mM (●); 1.8 mM (●). B, double-reciprocal plots of transferase activity at various concentrations of pantetheine and different fixed concentrations of malonyl-CoA: 0.993 mM (○); 0.276 mM (●); 0.463 mM (○). C, double-reciprocal plots of the K+ for malonyl-CoA (○) and pantetheine (●) against concentrations of pantetheine and malonyl-CoA, respectively. Units of K+ and S are millimolar and for V are units/mg of protein.
synthase the acceptor for the transferase (the ACP domain) is presumably always present at a concentration equal to that of the acyl-transferase; furthermore, unlike the model acceptors, the natural acceptor is localized close to the active site of the transferase.

Inhibitors—The transferase active site region includes the Gly-Xaa-Ser-Xaa-Gly motif common to the serine esterase group of enzymes, and several years ago it was demonstrated that one of the E. coli counterparts, the malonyl-CoA:ACP transacylase, is extremely sensitive to the serine esterase inhibitor phenylmethanesulfonyl fluoride (25, 26). The animal fatty acid synthase is also inhibited by phenylmethanesulfonyl fluoride, and although there has been one report indicating that it is the transferase that is modified (25), the effect is usually attributed to the sensitivity of the chain-terminating thioesterase to this inhibitor (27, 28). Indeed we found that the activity of the isolated transferase domain is completely unaffected by phenylmethanesulfonyl fluoride. On the other hand, activity could be completely abolished by certain thiol-directed reagents such as N-ethylmaleimide and methyl methanethiosulfonate. Protection from inhibition by N-ethylmaleimide was afforded by malonyl-CoA, but neither malonyl-CoA nor acetyl-CoA was effective in protecting the transferase from inhibition by methyl methanethiosulfonate. It is possible that these inhibitors react at two different sites, both of which are essential for transferase activity. Alternatively they may react at the same site but the differing sizes of substituent group introduced may affect the ability of substrate to protect. Methyl methanethiosulfonate introduces a small uncharged and non-hydrogen bonding CHS- group; N-ethylmaleimide, on the other hand, introduces a rather more bulky substituent group. It is possible then that whereas the binding of substrate at the active site serine can sterically prevent the binding of N-ethylmaleimide at the nearby thiol site, it cannot impede access by the smaller methyl methanethiosulfonate molecule. Neither iodoacetamide nor iodoacetate inhibited the transferase activity, perhaps because these reagents are sterically prevented from accessing the sensitive thiol. The transferase activity associated with the avian fatty acid synthase (29) and plant acetyl transferase have also been reported to be sensitive to sulfhydryl-directed reagents (30), whereas the E. coli malonyl transferase is reportedly insensitive (25). Further experimentation is required to identify the sensitive thiol group and determine its role, if any, in the catalytic process.

Interaction of the Transferase and ACP Domains—SDS-polyacrylamide gel electrophoresis of the isolated transferase and Edman degradation of the electrophoretically purified 47-kDa polypeptide had originally established the homogeneity of the fragment and its location in the fatty acid synthase subunit (17). However, subsequent analysis of the whole preparation of transferase revealed the presence of two small peptides originating from the ACP domain of the fatty acid synthase (details not shown); the small size of the ACP-derived peptides apparently precluded their detection by SDS-polyacrylamide gel electrophoresis. This observation suggested that regions of the ACP domain may have an affinity for the transferase domain. In an attempt to isolate transferase domains associated with intact ACP domains, we carried out the limited proteolysis under milder conditions and separated the transferase activity from the digest by gel filtration (see "Experimental Procedures" for details). This time the 47-kDa transferase species was associated with a smaller 8-kDa polypeptide. Amino-terminal sequencing of the 8-kDa species established that it began at residue Ala1210 of the parent fatty acid synthase polypeptide; this is the region that contains the ACP domain (16). When the isolated 47-kDa + 8-kDa complex was incubated with [2-14C]malonyl-CoA, radioactivity was found associated with both the 47- and 8-kDa species (Fig. 3). A relatively small amount of radioactivity was associated with a molecular species of 42 kDa, which most likely represents a truncated form of the transferase. Pretreatment of the preparation with iodoacetamide had no effect on loading of the transferase polypeptide with malonyl moieties but completely blocked the loading of the 8-kDa ACP polypeptide (Fig. 3). Since the transferase can load substrate in the absence of ACP, whereas ACP (E. coli) cannot load substrates in the absence of the transferase, the loading of the 8-kDa ACP species is most likely to occur via the transferase acyl-enzyme intermediate. The experiment confirmed the mutual affinity of the transferase and ACP domains and showed that malonyl translocation from the transferase domain to the ACP domain could be blocked by iodoacetamide. Since the ability of the transferase to translocate malonyl moieties from CoA thioester to pantetheine thioester is unaffected by iodoacetamide, the inability of the iodoacetamide treated ACP to act as an acceptor must have resulted from alkylation of the single thiol moiety present on the 8-kDa polypeptide, namely the 4'-phosphopantetheine thiol.

Thus the isolated transferase is competent to transfer substrate moieties to a model pantetheine acceptor and to the ACP domain derived from the multifunctional complex. To determine whether the 47-kDa polypeptide was capable of transferring substrate moieties to the intact fatty acid synthase subunit, we offered as an acyl acceptor the fatty acid synthase, both in the dimeric and monomeric form, under identical conditions. Malonyl-CoA was chosen as the acyl donor for these experiments since this substrate binds to only two sites on the fatty acid synthase, i.e. the 4'-phosphopantetheine thiol and the active site serine of the transferase (31, 32); thus binding to these two sites can be readily distinguished on the basis of the unique lability of the acyl-thioester

**Fig. 3. Isolation of a functional transferase-ACP complex from a tryptic digest of the fatty acid synthase core monomer.** The core monomers were trypsinized under mild conditions and fractionated by gel filtration. In one experiment the isolated transferase-ACP complex was treated with iodoacetamide (5 mM) prior to incubation with [2-14C]malonyl-CoA at room temperature for 10 min. Electrophoresis was performed on a 4–20% polyacrylamide gradient gel in the presence of SDS, the gel was then dried and exposed to x-ray film with intensifying screens for 48 h. Lane 1, molecular mass standards (values in kilodaltons); lane 2, isolated transferase-ACP complex (6.5 μg) treated with [2-14C]malonyl-CoA; lane 3, iodoacetamide-treated transferase-ACP complex (6 μg) incubated with [2-14C]malonyl-CoA.
to hydroxylamine treatment. The transferase preparation used for this experiment was prepared under mild conditions of proteolysis and contained the associated 8-kDa ACP domain. In order to avoid the possibility that transfer of acyl moieties from the 47-kDa transferase to the fatty acid synthase might be mediated via the noncovalently associated ACP polypeptide we blocked the sulfhydryl moiety of the ACP 4'-phosphopantetheine by prior treatment with iodoacetamide (Fig. 3). The transferase was first charged with [2-\textsuperscript{14}C]malonyl-CoA, then all traces of free malonyl-CoA were removed to eliminate the possibility of direct loading of substrate onto the fatty acid synthase subunit. The reaction of malonyl-transferase with fatty acid synthase, monomers or dimers, and the subsequent chromatography was carried out at 4 °C. Under these conditions, i.e. approximately 1 h in the cold, no significant change in the monomer/dimer equilibrium occurs (details not shown). When the monomers were presented as acceptors, approximately 70% of the radiolabeled malonyl moieties that were initially covalently linked to the transferase polypeptide were translocated to the fatty acid synthase subunit (Fig. 4). In contrast, when dimers were offered as acceptors only about 31% of the malonyl moieties were transferred to the fatty acid synthase. The translocation of malonyl moieties from the transferase to the fatty acid synthase was unaffected by the presence of a CoA scavenging system (ATP:citrate lyase, details not shown) and cannot therefore have occurred by unloading of the malonyl moieties to a CoA acceptor followed by independent reloading of the synthase monomer via the covalently associated transferase domain. Analysis of fractions from the Zorbax GF-250 HPLC column by SDS-polyacrylamide gel electrophoresis did not reveal the presence of 47-kDa polypeptides in the zone containing the fatty acid synthase (details not shown); thus the radioactive malonyl moieties present in these fractions must have been associated with the fatty acid synthase subunits. Indeed more than 80% of the radioactivity transferred to the fatty acid synthase was labile to hydroxylamine treatment, indicating linkage via a thioester, rather than an oxygen ester as in the case of the malonyl-transferase. Since the only thiol group of the fatty acid synthase capable of binding malonyl moieties is the 4'-phosphopantetheine site (31, 32), the malonyl moieties must have been covalently linked to the ACP domain of the synthase. In these experiments equimolar concentrations (1.4 μM) of fatty acid synthase monomer or dimer were used so that the actual concentration of ACP acceptor was twice as high in the case of the dimer. Despite this potential advantage favoring the dimer, the monomer was a more effective acceptor. This result suggests that access of the 47-kDa transferase to the 4'-phosphopantetheine of the fatty acid synthase is impeded in the dimer because of the close juxtaposition of the pantetheine of one subunit with domains of the opposing subunit (16). These findings lent support to the hypothesis that substrate loading from CoA ester to the 4'-phosphopantetheine of one subunit proceeds via intermediate covalent attachment to the transferase active site of the adjacent subunit.

These experiments indicate that the functional interaction between the transferase and ACP components of the multifunctional complex is supported by a strong noncovalent interaction between these domains. Since, in the head-to-tail arrangement of the two fatty acid synthase subunits, the transferase domain (located near the amino terminus) of one subunit is positioned close to the ACP domain (located near the carboxyl terminus) of the opposing subunit, it is likely that the contacts formed between these domains contribute to the stabilization of the dimer.

The strategy of exposing new potential sites of limited proteolysis that are protected at the subunit interface, by first dissociating the dimer into its subunits, may be useful in dissecting out the remaining functional domains from the core of the multifunctional protein. This is the first report describing the isolation of a transferase capable of translocating both acetyl and malonyl moieties between CoA and ACP thioester forms. The naturally occurring multifunctional transferases isolated previously from plant and bacterial sources exist in two discrete forms, one specific for malonyl transfer, the other for acetyl transfer that range in size from 30–48 kDa. The combination of these two functions in a single domain of 47 kDa provides yet another example of the remarkable parsimonious nature of the multifunctional animal fatty acid synthase.

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