Characterization of the malonyl-/acetyltransacylase domain of the multifunctional animal fatty acid synthase by expression in Escherichia coli and refolding in vitro

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cDNAs of various lengths encoding the second domain of the multifunctional fatty acid synthase (FAS) have been expressed in Escherichia coli and the recombinant proteins refolded in vitro to catalytically active monomeric malonyl-/acetyltransacylases. FAS residues 428–487, previously thought to represent the amino terminus of the malonyl-/acetyltransacylase, can be omitted from the recombinant enzyme with no loss in catalytic activity. This shortened transacylase, consisting of FAS residues 488–809, can be repeatedly denatured and renatured in vitro with reproducibly high recovery and no loss in specific activity. When expressed as a soluble enzyme in Spodoptera frugiperda cells, this transacylase has the same specific activity as the enzyme that has been refolded in vitro. The refolded transacylase consisting of FAS residues 488–809, but not the longer enzyme consisting of residues 428–815, can be crystallized readily. These results suggest that FAS residues 428–487, previously thought to represent the amino terminus of the malonyl/acetyltransacylase, are not required for catalysis of the transacylase reaction. This region of the FAS is less well conserved than the transacylase catalytic domain and may constitute an extended structural linker that facilitates the functional interaction between the transacylase and acyl carrier protein domains.

Keywords: amino terminal truncation/crystallization/refolding/Sf9 cells

Introduction

The animal fatty acid synthases (FASs) consist of two identical polypeptides, each carrying six enzymes and an acyl carrier protein, that are juxtaposed to form two centers for the synthesis of palmitic acid from acetyl- and malonyl-CoA. The amino terminal domain, encompassing approximately the first 406 residues of the 2505-residue multifunctional polypeptide, constitutes the β-ketoacyl synthase (Amy et al., 1989, Holzer et al., 1989, Schweizer et al., 1989, Witkowski et al., 1991). Residues 428–815 have been assigned to the second domain, which catalyzes the transfer of both acetyl and malonyl moieties from CoA ester to enzyme-bound form (Witkowski et al., 1991; Rangan and Smith, 1996). The intervening proline-rich sequence of approximately 20 residues is thought to constitute an interdomain linker region (Smith, 1994).

Although the transacylase component of the multifunctional FAS and its Escherichia coli counterpart share significant sequence similarity, they differ in several respects. Thus the  E.coli enzyme, typical of that found in plants and most microorganisms, exists as a discrete monofunctional polypeptide that catalyzes exclusively the transfer of malonyl moieties from CoA to acyl carrier protein thioester (Ruch and Vagelos, 1973); in E.coli the recruitment of acetyl moieties from acetyl-CoA for fatty acid synthesis is attributed to a specialized β-ketoacyl synthase III that catalyzes only the initial condensation reaction (Jackowski and Rock, 1987; Jackowski et al., 1989). Furthermore, the amino terminus of the E.coli malonyl transacylase aligns with residue 489 of the rat FAS indicating that the region extending from residues 428 to 488 of the multifunctional FAS has no counterpart in FASs that are composed of monofunctional polypeptides (Rangan and Smith, 1996). The primary goal of the present study was to determine whether residues 428–488 of the rat FAS play an essential role in catalysis of either the malonyl or acetyl transfer reactions.

Materials and methods

Materials

The pET17b and pET23a expression vectors were obtained from Novagen (Madison, WI). [1-14C]acetyl-CoA (54 Ci/mol) and [2-14C]malonyl-CoA (57 Ci/mol) were purchased from Moravek Biochemicals (Brea, CA). Rabbit anti-(rat)-FAS antibodies were prepared as described previously (Smith, 1973) and purified by affinity chromatography on Sepharose-antigen columns, essentially as recommended by Pharmacia LKB. Alkaline phosphatase-coupled goat anti-rabbit IgG antibodies and SDS–PAGE standards were purchased from Bio-Rad (Hercules, CA). Molecular mass standards for gel filtration chromatography were obtained from Sigma Chemical (St Louis, MO). Sf9 (Spodoptera frugiperda) cells were obtained from ATCC (Rockville, MD) and FASTBACK kit, Grace’s insect medium and other insect cell culture medium components from Life Technologies (Grand Island, NY). Synthetic oligonucleotides were obtained from Operon Technologies (Alameda, CA).

Construction of transacylase expression plasmids

Construction of transacylase expression plasmids pET17b/436-MAT and pET17b/388-MAT has been described previously. The pFAS54, which consists of nucleotides 642–2544 of the FAS cDNA (Amy et al., 1989), was used as template DNA for generation by polymerase chain reaction (PCR) of constructs of different lengths all encoding the transacylase. The conditions used for PCR amplification of DNA were described earlier (Rangan and Smith, 1996). Amplified products were purified, digested with restriction enzymes NdeI and EcoRI and cloned into the pET23a expression vector restricted with appropriate enzymes. The plasmids encoding transacylases of different lengths are referred to as pET23a/382-MAT (residues 429–809 of FAS), pET23a/349-MAT (residues 468–815 of FAS), pET23a/329-MAT (residues 488–815 of FAS) and pET 23a/323-MAT (residues 488–809 of FAS). E.coli BL21(DE3) cells
were used for transformation and expression of the recombinant proteins was induced by 1 mM isopropyl-β-d-thiogalactoside.

Expression of the recombinant transacylases in *E. coli* and their renaturation from inclusion bodies and purification have been described earlier (Rangan and Smith, 1996).

**Expression of recombinant transacylase in *Sf*9 insect cells**

The PCR-amplified DNA corresponding to residues 488–809 of FAS was digested with *XbaI/NotI* and cloned into the modified pFASTBAC 1 transfer vector restricted with appropriate enzymes. The authenticity of the DNA was confirmed by DNA sequencing. This construct was used to generate recombinant baculoviral stocks by the transposition method using a FASTBACK kit. *Sf*9 cells were then infected with recombinant baculoviral stocks and grown for 48 h. Cells were pelleted and homogenized in 5 vol. of buffer A, which consisted of 50 mM Tris–HCl buffer (pH 7.0), 1 mM EDTA, 1 mM phenylmethanesulfonyl fluoride, 1 mM DTT and 10% glycerol. The homogenate was centrifuged at 16 000 r.p.m. for 60 min and the supernatant was used for purification of recombinant transacylase.

**Purification of cytosolic recombinant transacylase**

Cytosol from *Sf*9 insect cells was applied at 0–4°C to a column of DE-53 anion exchanger equilibrated with buffer A. The column was washed with buffer A and transacylase activity eluting in the unbound protein fraction was collected. This material was dialyzed overnight against buffer B, consisting of 50 mM Tris–HCl buffer (pH 8.0), 1 mM EDTA, 1 mM DTT and 10% glycerol, and applied to the same DE-53 ion-exchange column that had been equilibrated with buffer B. The column was washed extensively with buffer B, then bound proteins were eluted with an 800 ml gradient of 0–500 mM NaCl in buffer B. Transacylase activity eluted between 100 and 120 mM NaCl. Fractions containing the enzyme were pooled and concentrated using a Centriplus-10 apparatus (Amicon). Recombinant transacylase was further purified by high-performance liquid chromatography (HPLC) using an Ultrasphergel SEC3000 (30 cm × 7.5 mm i.d.) gel filtration column pre-equilibrated with 250 mM potassium phosphate buffer (pH 7.5), 1 mM EDTA and 1 mM DTT. Fractions containing transacylase activity were pooled, glycerol was added to a final concentration of 10% and the mixture was stored at −70°C.

**Electrophoretic analysis and Western blotting**

SDS–PAGE was performed using 12% polyacrylamide gels. Western blot analysis employed monospecific rabbit anti-(rat)-FAS and alkaline phosphatase-coupled goat anti-rabbit IgG antibodies as the primary and secondary antibodies, respectively.

**Electrospray ionization mass spectrometry**

Electrospray ionization mass spectrometry (Smith et al., 1990) was performed on a VG BioQ triple-quadrupole electrospray mass spectrometer (Micromass, Altrincham, UK) interfaced with a Michrom narrow-bore HPLC system (Michrom BioResources, Auburn, CA). The mass spectrometer was controlled and data were analyzed using software obtained from Micromass (Ferrige et al., 1992). Protein (200–250 pmol) was desalted by reversed-phase narrow-bore HPLC and delivered to the mass spectrometer at a flow rate of 4 ml/min. External calibration of the mass scale was based upon multiply protonated ions derived from horse heart myoglobin (16 951.4 Da).

Assay of transacylase activity

Transacylase activity was determined at 0°C using either [1-14C]malonyl-CoA or [2-14C]malonyl-CoA as substrate and pantetheine as model acceptor as described previously (Rangan and Smith, 1996). A unit of activity is the amount of enzyme catalyzing the utilization of 1 μmol of substrate per minute.

**Gel filtration chromatography**

An Ultraspherogel SEC3000 (7.5 mm × 30 cm) HPLC gel filtration column, equilibrated with 250 mM potassium phosphate buffer (pH 7.5), 1 mM EDTA and 1 mM DTT at a flow rate of 1 ml/min, was used to determine the native molecular mass of the recombinant transacylases. Alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa) and carbonic anhydrase (29 kDa) were used as standards; they had retention times of 9.45, 9.89, 10.4 and 10.9 min, respectively.

**Crystallization of the malonyl-acetyltransacylase**

The malonyl-acetyltransacylase corresponding to residues 488–809 of the FAS was expressed, purified and concentrated to 8 mg/ml. Crystallization conditions were explored by the standard hanging drop method, using the Hampton Research crystal screen at 20°C. Droplets were composed of 2 μl of reservoir solution and 2 μl of enzyme solution.

**Results**

**Truncation of the 388-residue transacylase**

Recently, we have shown that it is possible to express, as an active recombinant protein, part of the FAS (residues 384–815) that previously had been obtained as a catalytically active fragment by proteolysis (Rangan and Smith, 1996). Residues 384–428 (full-length FAS numbering) could be eliminated from the recombinant protein without loss of activity, consistent with the hypothesis that this region, which is relatively rich in proline residues and is poorly conserved among multifunctional polyketide synthases (PKSs) and FASs, may constitute an interdomain linker (Rangan and Smith, 1996). Identification of the *E. coli* malonyl ACP (acyl carrier protein) transacylase with transacylase segments from multifunctional PKSs and FASs revealed that, although these proteins are all clearly structurally related (Rangan and Smith, 1996), the amino terminus of the *E. coli* enzyme aligns with residue 489 of rat FAS, which is located some 60 residues downstream from the presumed amino terminus of the transacylase domain (Figure 1). In order to ascertain whether these additional 60 residues were essential either for correct folding of the transacylase domain, for catalysis of the transfer reaction or for determining the dual substrate specificity of the enzyme, we deleted amino terminal sequences from the 388 residue transacylase. Deletions were not made beyond the region that aligned with the amino terminus of the *E. coli* enzyme, since a structurally important β-sheet is located at residues 5–8 in this enzyme (Serré et al., 1995). In addition, we deleted several residues from the carboxy terminus that also appear to have no counterparts in the *E. coli* malonyl transacylase. Thus, Asp809 was chosen to be the carboxy terminus of the rat enzyme since, in multiple alignments of different transacylases, it aligns close to the carboxy terminus of the monofunctional, malonyl-specific enzymes. In multifunctional FASs and PKSs, Asp809, or its equivalent residue, is followed immediately by a proline-rich region that exhibits the characteristics of an interdomain linker.
Expression and refolding of malonyl-/acetiltransacylase

Fig. 1. Alignment of the carboxy terminal region of the β-ketoacyl synthase domain, the amino terminal region of the malonyl-/acetiltransacylase domain and the intervening linker region of the rat FAS with homologous regions of other multifunctional FASs, PKSs and monofunctional β-ketoacyl synthases and malonyltransacylases. The Clustal W program was used to construct the multiple alignment (Thompson et al., 1994). FAS, fatty acid synthase; MAS, mycocerosic acid synthase from Mycobacterium tuberculosis; DEBS, 6-deoxyerythronolide B synthase from Saccharopolyspora erythraea; KSI, β-ketoacyl synthase I; H. influ., Hemophilus influenzae; MT, malonyltransacylase. The positions of universally conserved residues are marked d and those that accommodate only conservative mutations are marked s. Gaps introduced in the alignment are shown as dashes. GenBank accession numbers for the sequences are as follows: rat FAS, M76767 X14175; chicken FAS, J03860 M22987; human FAS, U26644; M. tuberculosis MAS, M95808; S. erythraea DEBS, M63676 M63677; E. coli KSI, M24427; H. influenzae KSI, U32829 L42023; barley KSI, M95172; castor KSI, L13242; E. coli MT, M87040; H. influenzae MT, U32701 L42023.

The constructs representing residues 429–809, 468–815, 488–815 and 488–809 of the rat FAS, plus an amino terminal methionine residue, were engineered and the encoded recombinant proteins were overexpressed in E. coli BL21(DE3) cells. In all cases, most of the recombinant protein was found in the inclusion body pellet and only a very small proportion was found in the cytosol. We solubilized the proteins present in inclusion bodies using 7 M urea, subjected them to the renaturation process and purified them to >95% purity. An electrophoretic analysis of the purified recombinant proteins is shown in Figure 2; all purified proteins were recognized by rabbit anti-rat liver FAS antibodies. The experimentally determined molecular mass of the 388-residue protein was exactly as predicted for the full-length polypeptide (Table I). However the molecular masses obtained for the 436- and 323-residue proteins matched the values predicted for polypeptides lacking the amino terminal methionine residues (Table I), consistent with these residues having been removed by the E. coli methionylaminopeptidase, an enzyme that exhibits high activity toward polypeptides containing serine or alanine in

Fig. 2. Electrophoretic analysis of purified recombinant transacylases. (A), SDS–PAGE. Gels were stained with coomassie brilliant blue R-250. Lane 1, molecular mass standards; lanes 2–7, purified 436, 388, 382, 349, 329 and 323 residue transacylases, respectively. (B) Western blot analysis of transacylases subjected to SDS–PAGE. Lane 1, prestained molecular mass standards; lanes 2–7, purified 436, 388, 382, 349, 329 and 323 residue transacylases, respectively.

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The transacylases of different lengths exhibited very similar specific activities with acetyl-CoA as substrate (Table I). The transacylases of usually such aggregation is strongly dependent on protein length. In an attempt to validate further the approach of using inclusion bodies as active, full-length FAS (Joshi and Smith, 1993). Thus, we sought, for comparison, an alternative expression system similarity to the 309-residue enzyme that is able to translocate both acetyl and malonyl moieties. The transacylase was purified to about 90% purity by ion-exchange chromatography and gel filtration with an overall yield of 21%. The specific activity of the purified cytosolic commonly encountered in refolding proteins solubilized from inclusion bodies is the tendency of these proteins to aggregate; usually such aggregation is strongly dependent on protein concentration. We therefore performed a series of denaturation and refolding experiments, in triplicate, starting with three different protein concentrations of previously refolded 323-residue transacylase. The specific activity of the refolded enzyme was reproducible to better than ±5%. The overall recoveries of soluble protein were also very good, averaging 80%, and the final specific activity was independent of protein concentration. This experiment shows clearly that the renaturation process occurs readily and reproducibly and that the same enzyme preparation can be repeatedly denatured and refolded with full retention of catalytic activity.

In an attempt to validate further the approach of using renatured protein as a source of catalytically active transacylase, we sought, for comparison, an alternative expression system that might directly provide soluble recombinant protein that had been folded in vivo.

Properties of the 323-residue transacylase refolded in vivo using Sf9 cells

The Sf9/baculoviral expression system was chosen since it has been used successfully in our laboratory to produce catalytically active, full-length FAS (Joshi and Smith, 1993). Thus, Sf9 insect cells were infected with a recombinant baculovirus encoding the 323-residue transacylase protein. In contrast to our experience with E.coli cells, we found that the recombinant protein was located entirely in the cytosol fraction of Sf9 cells. The transacylase was purified to about 90% purity by ion-exchange chromatography and gel filtration with an overall yield of 21%. The specific activity of the purified cytosolic

### Table I. Activities of truncated forms of transacylase

<table>
<thead>
<tr>
<th>Number of residues</th>
<th>Location in FAS</th>
<th>Determined molecular mass (Da)</th>
<th>Calculated molecular mass (Da)</th>
<th>Specific activity&lt;sup&gt;a&lt;/sup&gt; (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>436&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(MASM)&lt;sup&gt;386&lt;/sup&gt;RGGL...PNAL&lt;sup&gt;315&lt;/sup&gt;</td>
<td>47 091</td>
<td>47 100&lt;sup&gt;6&lt;/sup&gt;</td>
<td>2.95 ± 0.08</td>
</tr>
<tr>
<td>388&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(MI)&lt;sup&gt;386&lt;/sup&gt;RTME...PNAL&lt;sup&gt;315&lt;/sup&gt;</td>
<td>42 467</td>
<td>42 469</td>
<td>2.64 ± 0.08</td>
</tr>
<tr>
<td>382&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(MI)&lt;sup&gt;386&lt;/sup&gt;RTME...TGD&lt;sup&gt;399&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>2.49 ± 0.09</td>
</tr>
<tr>
<td>349&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(MI)&lt;sup&gt;408&lt;/sup&gt;RGYT...PNAL&lt;sup&gt;315&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>3.19 ± 0.05</td>
</tr>
<tr>
<td>329&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(MI)&lt;sup&gt;418&lt;/sup&gt;SQRP...PNAL&lt;sup&gt;315&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
<td>2.39 ± 0.10</td>
</tr>
<tr>
<td>323&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(MI)&lt;sup&gt;429&lt;/sup&gt;SQRP...TGD&lt;sup&gt;399&lt;/sup&gt;</td>
<td>35 336</td>
<td>35 338&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.63 ± 0.10</td>
</tr>
</tbody>
</table>

Amino acids in parentheses are contributed by the expression vector and do not originate from the FAS.
<sup>a</sup>Substrate is acetyl-CoA.
<sup>b</sup>Amino acids in parentheses are contributed by the expression vector and do not originate from the FAS.
<sup>c</sup>Excludes amino terminal methionine.
<sup>d</sup>Not determined.

### Table II. Kinetic parameters of recombinant transacylases

<table>
<thead>
<tr>
<th>Transacylase length</th>
<th>Acetyl-CoA</th>
<th>Malonyl-CoA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ (µM)</td>
<td>$V_{max}$ (U/mg)</td>
</tr>
<tr>
<td>436 residues&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.53 ± 0.39</td>
<td>3.16 ± 0.08</td>
</tr>
<tr>
<td>388 residues&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.92 ± 0.43</td>
<td>2.69 ± 0.08</td>
</tr>
<tr>
<td>382 residues&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.10 ± 0.50</td>
<td>2.60 ± 0.09</td>
</tr>
<tr>
<td>349 residues&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.02 ± 0.55</td>
<td>3.46 ± 0.06</td>
</tr>
<tr>
<td>329 residues&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.44 ± 0.05</td>
<td>2.35 ± 0.03</td>
</tr>
<tr>
<td>323 residues&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.74 ± 0.10</td>
<td>2.65 ± 0.05</td>
</tr>
</tbody>
</table>

$K_m$ and $V_{max}$ values for acetyl-CoA and malonyl-CoA substrates were determined using 3 mM pantetheine as the acceptor.
<sup>a</sup>Rangan and Smith (1996).

### Table III. Reproducibility of the renaturation procedure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{320}$ before denaturation</td>
<td>0.434</td>
<td>0.668</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Protein recovered (%)</td>
<td>74.3 ± 2.2</td>
<td>76.6 ± 3.1</td>
<td>84.4 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Activity recovered (%)</td>
<td>79.9 ± 2.0</td>
<td>83.0 ± 4.0</td>
<td>85.9 ± 5.1</td>
<td></td>
</tr>
<tr>
<td>Specific activity before denaturation</td>
<td>2.81</td>
<td>2.70</td>
<td>2.85</td>
<td></td>
</tr>
<tr>
<td>Specific activity after renaturation</td>
<td>3.02 ± 0.14</td>
<td>2.92 ± 0.08</td>
<td>2.90 ± 0.14</td>
<td></td>
</tr>
</tbody>
</table>

The denaturation and renaturation procedures were carried out in triplicate. Values represent means ± standard deviation.
Expression and refolding of malonyl-/acetyltransacylase

Fig. 3. Crystals of 388-residue malonyl-/acetyltransacylase. The plateshaped crystals measure ~1.5 x 0.05 mm.

protein was 2.68 ± 0.13 U/A280, which is similar to that of the enzyme folded in vitro. This result indicates that in vitro renaturation of the malonyl-/acetyltransacylase recovered from inclusion bodies generates a protein with the same biological activity as that which is refolded in a eukaryotic cell environment.

Crystallization of the malonyl-/acetyltransacylase

Exhaustive attempts to crystallize the 388-residue malonyl-/acetyltransacylase, corresponding to FAS residues 428–815, were unsuccessful. In contrast, crystals of the 322-residue enzyme corresponding to FAS amino acids 488–809 of the FAS were readily obtained under a variety of conditions, including: 2.0 M ammonium sulfate, 0.1 M Tris–HCl (pH 8.5); 4.0 M sodium formate; 1.4 M sodium citrate, 0.1 M Hepes (pH 7.5); and 2.0 M ammonium sulfate, 2% (v/v) polyethylene glycol 400, 0.1 M Hepes (pH 7.5). The largest crystals were obtained using 1.4 M sodium citrate/0.1 M Hepes (pH 7.0–8.5) with the addition of 10% glycerol to the reservoir solution. The crystals grew as long thin plates, frequently in bunches (Figure 3).

This observation suggests that the sequences removed by amino terminal truncation may have inhibited crystallization of the longer protein, perhaps because of high flexibility in this region. Truncation of other proteins has been used successfully to good quality crystals of the catalytic domains, notably dihydrolipoyl transacylase (Mattevi et al., 1992) and phospholipase Cβ1 (Ellis et al., 1993, Essen et al., 1996). Conditions are currently being sought that will provide larger crystals of the malonyl-/acetyltransacylase that are suitable for X-ray analysis.

Analysis of recombinant transacylases by gel filtration chromatography

The subunit structure of the 436-, 388- and 323-residue malonyl-/acetyltransacylases were investigated by gel filtration HPLC under non-denaturing conditions. All of these proteins exhibited retention times in the range 10.8–11.0 min, close to those of ovalbumin (45 kDa, 10.4 min) and carbonic anhydrase (29 kDa, 10.9 min), clearly indicating that the recombinant transacylases of various lengths all exist as monomers.

Discussion

In the multifunctional forms of FASs and PKSs, the transacylase domain is located on the carboxy terminal side of the β-ketoacyl synthase domain (Smith, 1994). The carboxy terminal boundary of the β-ketoacyl synthase domain has been identified by alignment with the conserved carboxy terminal region of β-ketoacyl synthase associated with monofunctional FAS systems, such as those found in plants and bacteria. In the multifunctional proteins, the carboxy terminus of the β-ketoacyl synthase domain is followed immediately by a region of ~17 residues that typically is rich in proline, often together with alanine and hydrophilic residues, particularly glutamate (Figure 1). This region is predicted to be a flexible, surface loop and previously was assigned the role of interdomain linker (Chang and Hammes, 1990, Smith, 1994).

However, the present study reveals that the following sequence of ~65 residues is not required for catalysis of the transacylase reaction, suggesting that it too may play a structural role, possibly as part of an extended linker region. The major distinguishing feature of this putative structural linker sequence (as represented by residues 405–487 in the rat FAS) is that it is less well conserved among the FASs than is the essential catalytic region (as represented by residues 488–809 in the rat FAS). Whereas the essential catalytic domain is 70% identical (89% similar, if conservative replacements are included) in rat, human and chicken FASs, the putative linker is only 39% identical (65% similar). One might argue that this feature is consistent with residues 405–487 playing a structural, rather than a catalytic role. Why is such a long linker sequence present between the β-ketoacyl synthase domain and malonyl-/acetyltransacylase catalytic domains? According to the generally accepted mechanism for the animal FAS, the β-ketoacyl synthase and malonyl-/acetyltransacylase are not required to communicate directly with each other. Thus the transfer of acetyl moieties from Ser-581 in the transacylase domain to Cys-161 in the β-ketoacyl synthase domain proceeds via a 4'-phosphopantetheine intermediate. Although there is circumstantial evidence suggesting that it is the 4'-phosphopantetheine attached to Ser-2151 of the ACP domain associated with the opposite subunit that accepts the acetyl (and malonyl) moiety from Ser-581 (Petithory and Smith, 1993), the possibility that the 4'-phosphopantetheine associated with the same subunit can accept acetyl moieties from the transacylase domain has not yet been completely excluded. It is conceivable therefore that the long linker sequence may be required to position the active-site Ser-581 residue close to the 4'-phosphopantetheine residue attached to Ser-2151 of either the same subunit and/or the companion subunit. It is also possible that the extended linker region might directly facilitate interaction with the 4'-phosphopantetheine moiety by providing a ‘docking site’ for the ACP domain. Experiments designed to test these possibilities are under way.

The finding that the region of the FAS polypeptide required for catalysis of the malonyl-/acetyl transacylation reactions can be shortened to approximately the length of the E. coli malonyl transacylase, together with the observation that catalytically important serine and histidine residues are positionally conserved in these enzymes, strongly suggests that, despite their differences in substrate specificity, these enzymes may share similar three-dimensional features. Thus the availability of a crystal structure for the E. coli enzyme (Serre et al., 1995) could be extremely useful in elucidating structural features that contribute to the catalytic properties and substrate specificity of the mammalian FAS homolog.

The multifunctional FASs and PKSs share a number of common features in the organization of functional domains.
within the polypeptides, as well as in the catalytic mechanism and amino acid sequences of the constituent domains. Nevertheless, several distinguishing features are beginning to emerge that appear to have important implications so far as the subunit organization and overall reaction mechanism is concerned. For example, whereas all FAS subunits consist of a complete biosynthetic module consisting of a β-ketoacyl synthase, transacylase, dehydrase, enoyl reductase, β-ketoreductase, ACP and thioesterase, PKSs may contain more than one biosynthetic module per subunit and some of the biosynthetic modules are incomplete, lacking one or more of the β-carbon processing enzymes (Katz and Donadio, 1993). Thus in the FASs, the fatty acid product is formed as the result of iterative use of the same biosynthetic module for each of the elongation steps, whereas in the PKSs the polyketide product is formed as the result of the sequential participation of different biosynthetic modules for each of the elongation steps. Recently, a structural model has been proposed to account for the sequential use of the various biosynthetic modules present in a multifunctional PKS (Staunton et al., 1996). In this model, each biosynthetic module forms a parallel dimer that is stabilized by homodimeric interactions between the β-ketoacyl synthases, malonyl transacylases, ACPs and thioesterases of adjacent subunits. The model is supported by the observation that all of the of biosynthetic modules and certain individual domains can be isolated as homodimers. In contrast, although the FAS biosynthetic modules, or subunits, are isolated as homodimers, none of the individual catalytic domains has been isolated as a dimer. For example, the molecular mass of the thioesterase domain isolated from the rat FAS is 32 300 Da by equilibrium sedimentation and 35 000 Da by SDS–PAGE (Dileepan et al., 1978). As shown in the present study, the malonyl–acetyltransacylase domain, expressed as a recombinant protein in E.coli and refolded to a catalytically active protein in vitro, also exists as a monomer. These observations are consistent with the original proposal that the FAS is composed of antiparallel oriented dimers (Stoops and Wakil, 1981), rather than parallel oriented dimers, as recently proposed for the PKSs (Staunton et al., 1996).

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References


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