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Fatty Acid Synthase, A Proficient Multifunctional Enzyme†

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The animal fatty acid synthase is the most sophisticated entry in the newly recognized class of multifunctional enzymes. The subunit protein of this elegant multienzyme has a molecular weight of 260 000 and contains, in separate domains, seven different catalytic activities and a site for the prosthetic group, 4′-phosphopantetheine, of the acyl carrier protein. Investigations of fatty acid biosynthesis not only yielded information about this multienzyme system but uncovered significant concepts basic to biochemistry at large. For instance, the notion that synthetic pathways are the reversal of degradative reactions was refuted in part by studies on fatty acid biosynthesis (Wakil et al., 1957). It is now well accepted that anabolic pathways may not be the same as the degradative pathways. Also, the role of the vitamin biotin in biological reactions was first recognized in studies of fatty acid synthesis by the discovery of biotin as a prosthetic group of acetyl-CoA carboxylase (Wakil et al., 1958). Several such biotin-containing enzymes have since been recognized and studied. Moreover, the activation of acetyl-CoA carboxylase by citrate (Waite & Wakil, 1962; Martin & Vagelos, 1962) provided one of the early examples that led to the formulation of the model for allosteric modification of proteins by Monod, Wyman, and Changeux (1965). The finding that a protein, acyl carrier protein (ACP), acts as a coenzyme was first demonstrated in enzymatic reactions involved in fatty acid synthesis (Majerus et al., 1964; Wakil et al., 1964). This protein binds substrates and all acyl intermediates as thioesters and channels them into the synthetic pathway. Finally, studies of this system (Knobling et al., 1975; Stoops et al., 1975) led to the recognition of multifunctional proteins as a new class of enzymes with two or more catalytic domains associated with a single polypeptide (Kirschner & Bisswanger, 1976). This multifunctional protein is encoded by a single gene which may have evolved by fusion of component genes. This review presents our current knowledge of this multifunctional enzyme and provides a summary of the organization of component activities on the protein and their function in the synthesis of long-chain fatty acids.

Long-chain fatty acids are essential constituents of membrane lipids and are important substrates for energy metabolism of the cell. Palmitate, the most abundant acid, is synthesized de novo from acetyl-CoA, malonyl-CoA, and NADPH by the fatty acid synthase according to the following reaction:

$$CH_3COS\text{–}CoA + 7HOOCCH_2COS\text{–}CoA + 14NADPH + 14H^+ \rightarrow CH_3CH_2(CH_2CH_2)_6CH_2COOH + 14NADP^+ + 6H_2O + 8CoA\text{–}SH + 7CO_2 \quad (1)$$

Basically, this reaction consists of elongating the acetyl group by C_2 units derived from malonyl-CoA in a stepwise and sequential manner. For instance, in the synthesis of palmitate there are over 40 steps with at least 30 acyl intermediates.

The nature of these reactions and the intermediates involved became known primarily from studies of fatty acid synthesis in cell-free extracts of *Escherichia coli* (Wakil, 1970; Volpe & Vagelos, 1977; Bloch & Vance, 1977). A protein known as acyl carrier protein (ACP), with its 4′-phosphopantetheine prosthetic group (M_r 8847), was identified as the coenzyme that binds all acyl intermediates as thioester derivatives. The individual enzymes were then isolated and utilized in the reconstitution of the synthesis pathway. The following enzymes and reactions are involved in the synthesis of palmitate:

acetyl transacylase

$$CH_3COS\text{–}CoA + ACP\text{–}SH \rightleftharpoons CH_3COS\text{–}ACP + CoA\text{–}SH \quad (2)$$

malonyl transacylase

$$HOOCCH_2COS\text{–}CoA + ACP\text{–}SH \rightleftharpoons HOOCCH_2COS\text{–}ACP + CoA\text{–}SH \quad (3)$$

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β-ketoacyl synthase (condensing enzyme)

$$CH_3COS\text{–}ACP + \text{synthase–SH} \rightleftharpoons CH_3COS\text{–synthase} + \text{ACP–SH} \quad (4)$$

$$CH_3COS\text{–synthase} + HOOCCH_2COS\text{–ACP} \rightarrow CH_3COCH_2COS\text{–ACP} + CO_2 + \text{synthase–SH} \quad (5)$$

β-ketoacyl reductase

$$CH_3COCH_2COS\text{–ACP} + NADPH + H^+ \rightleftharpoons D\text{-}CH_3CHOHCH_2COS\text{–ACP} + NADP^+ \quad (6)$$

β-hydroxyacyl dehydratase

$$D\text{-}CH_3CHOHCH_2COS\text{–ACP} \rightleftharpoons \textit{trans}\text{-}CH_3CH{=}CHCOS\text{—ACP} + H_2O \quad (7)$$

enoyl reductase

$$\textit{trans}\text{-}CH_3CH{=}CHCOS\text{—ACP} + NADPH + H^+ \rightarrow CH_3CH_2CH_2COS\text{–ACP} + NADP^+ \quad (8)$$

thioesterase

$$CH_3CH_2(CH_2CH_2)_6CH_2COS\text{–ACP} + H_2O \rightarrow CH_3CH_2(CH_2CH_2)_6CH_2COOH + \text{CoA–SH} \quad (9)$$

These reactions are essentially the same in all organisms. A malonyl group derived from malonyl-CoA is condensed with an acetyl group, as a primer, with loss of carbon dioxide (reactions 4 and 5). The β-ketoacyl derivative is reduced in three consecutive steps (reactions 6, 7, and 8) to the saturated acyl derivative, which then acts as a primer for further elongation and reduction cycles to yield ultimately a palmitoyl derivative. The latter is either hydrolyzed to free palmitate as in bacteria and animal tissue (reaction 9), transferred to CoA–SH to form palmitoyl-CoA as in yeast (Schreckenbach et al., 1977), or utilized directly in the synthesis of phosphatidic acid as in *E. coli* (Rock et al., 1981).

In prokaryotes and plants, the enzymes of fatty acid synthesis can be readily separated by conventional procedures (Wakil, 1970; Volpe & Vagelos, 1977; Stumpf, 1984). In yeast and animal cells the enzyme activities purify together and were initially thought to be tightly associated complexes. However, later investigations clearly showed that yeast (Schweizer et al., 1975; Stoops et al., 1978) and animal (Stoops et al., 1975) fatty acid synthases are multifunctional in nature. As multifunctional enzymes, the catalytic sites are arranged as a series of connected globular domains. In yeast and other fungi, the enzyme activities are distributed on two separate subunits: subunit α (M_r 207 863), which contains an attachment site of the prosthetic group of ACP, 4′-phosphopantetheine, and domains for two enzyme activities, β-ketoacyl reductase and β-ketoacyl synthase (Mohamed et al., 1988); subunit β (M_r 220 077), which contains domains for the remaining five enzymes, the acetyl transacylase, the enoyl reductase, the dehydratase, and the malonyl/palmitoyl transacylases (Chirala et al., 1987). The active enzyme is an $\alpha_6\beta_6$ complex with a molecular weight of 2.4×10^6 (Schweizer et al., 1975; Stoops et al., 1975, 1978; Lynen, 1980).

In animal cells the component enzymes of fatty acid synthesis are covalently linked on a single polypeptide chain. The native enzyme (M_r ~500 000) isolated from animal cells consists of two such multifunctional polypeptides (Arslanian et al., 1976; Buckner & Kolattukudy, 1976; Stoops et al., 1979; Smith et al., 1985). The mRNA coding for the avian (Zehner et al., 1980; Morris, 1982) and rat mammary gland (Mattick et al., 1981) synthases was shown to be a single contiguous mRNA which can be translated to a protein of M_r 260 000 that specifically binds to the anti-synthase antibodies and can be competed for by native synthase.

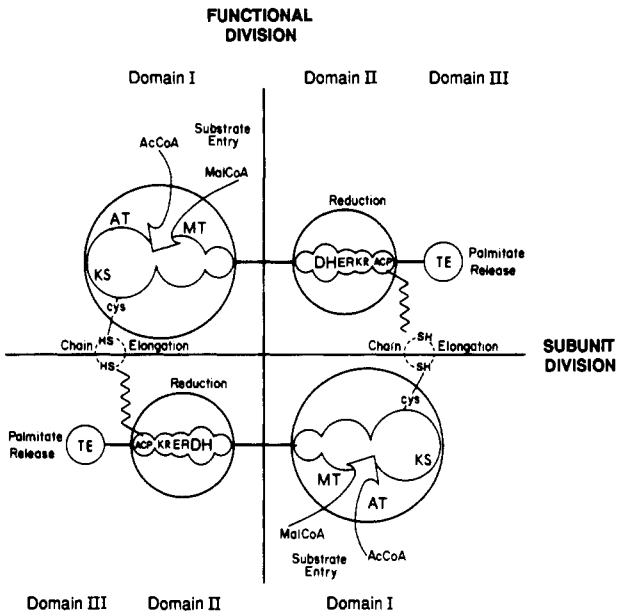


FIGURE 1: Functional map and organization of the subunit proteins of the chicken liver fatty acid synthase. The two subunits with their domains I, II, and III are drawn in an antiparallel arrangement (subunit division) so that two sites of palmitate synthesis are constructed (functional division). The abbreviations for the partial activities used are as follows: KS, β-ketoacyl synthase; AT, acetyl transacylase; MT, malonyl transacylase; DH, β-hydroxyacyl dehydratase; ER, enoyl reductase; KR, β-ketoacyl reductase; ACP, acyl carrier protein; TE, thioesterase. The wavy line represents the 4′-phosphopantetheine prosthetic group of ACP.

The organization of the various component activities on the subunit protein was determined after detailed proteolytic mapping of the synthase protein and subsequently confirmed by localization of their active sites in the primary sequence. Proteolytic cleavage of chicken liver fatty acid synthase by different proteases (trypsin, chymotrypsin, subtilisin, kallikrein, and *Myxobacter* protease) used either individually or in combination was performed, and the results were analyzed with respect to the kinetics and the size of fragments generated (Mattick et al., 1983a,b; Wong et al., 1983; Tsukamoto et al., 1983; Tsukamoto & Wakil, 1988). The results show that the fatty acid synthase subunit protein consists primarily of three major domains (I, II, and III) of M_r 127 000, 107 000, and 33 000, respectively. Each of these domains can be further subdivided by various proteases into a number of distinct regions that can be aligned and mapped. A proteolytic map was generated and served as a reference for the controlled cleavage of the fatty acid synthase, thereby allowing the isolation, determination, and placement of the functional activities within the subunit protein (Figure 1).

Chymotrypsin makes the most restricted cut of the synthase by hydrolyzing its subunits into two fragments of M_r 230 000 and 33 000. These fragments can be readily separated into pure fractions. The large fragment contains all the core activities of fatty acid synthesis, that is, acetyl and malonyl transacylases, β-ketoacyl synthase, β-ketoacyl and enoyl reductases, dehydratase, and ACP. The smaller fragment comprises the thioesterase activity and contains the COOH-terminal end of the synthase subunit (Mattick et al., 1983a). The properties of the isolated thioesterase were studied with long-chain acyl-CoA derivatives as substrates. The enzyme shows the highest rate of hydrolysis with palmitoyl-CoA but is also active with stearoyl-CoA and to a lesser extent with myristoyl-CoA (Lin & Smith, 1978; Libertini & Smith, 1979; Crisp & Wakil, 1982), reflecting the production of these acids

by the fatty acid synthase (Bressler & Wakil, 1961).

Cleavage of the synthase by other proteases made it possible to assign locations to the other component activities on the subunit protein. Domain I contains the NH_2 terminus of the polypeptide, the active cysteine–SH of the β-ketoacyl synthase, and the common serine–OH of the acetyl and malonyl transacylases (Joshi et al., 1970; Stoops & Wakil, 1982; Tsukamoto et al., 1983; McCarthy et al., 1983; Mikkelsen et al., 1985b). Hence, this domain functions as a site for entry of the substrates, the acetyl and malonyl groups, and their subsequent condensation to form carbon–carbon bonds (Figure 1). The β-ketoacyl derivative formed by this chain elongation is then reduced by the component enzymes associated with domain II, the reduction domain. In this domain the dehydratase and enoyl and β-ketoacyl reductases are located and ordered as shown in Figure 1 (Wong et al., 1983; Tsukamoto et al., 1983; Tsukamoto & Wakil, 1988). The ACP and its serine–OH for attachment of the 4′-phosphopantetheine cofactor are also located in domain II and connect the β-ketoacyl reductase (domain II) to the thioesterase (domain III).

This arrangement of the partial activities on the synthase subunit protein was recently confirmed by cloning and sequencing the cDNA of the component enzymes of chicken synthase (Chirala et al., 1989). In the predicted amino acid sequences, the active sites of the component activities were identified, and their location on the polypeptide confirmed the proteolytic mapping of the activities on the synthase subunit protein (Tsukamoto et al., 1983; Tsukamoto & Wakil, 1988). The complete amino acid sequence of the thioesterase domain of chicken liver synthase was determined by sequencing the protein (Yang et al., 1988) and confirmed by nucleotide sequencing of the cDNA (Kasturi et al., 1988; Yuan et al., 1988; Chirala et al., 1989). The amino acid sequence of the thioesterase of rat mammary gland synthase has also been predicted from nucleotide sequencing of the cDNA (Naggert et al., 1988) and shows about 70% homology to that of the thioesterase of chicken synthase. However, a much higher homology (~90%) was noted around the active serine sites and at the COOH-terminal ends of the two thioesterases, suggesting that these sequences are highly conserved and may be essential to the function of the thioesterase.

The complete amino acid sequence of the ACP domain prepared after tryptic digestion of the chicken liver fatty acid synthase has been determined (Huang et al., 1989) and confirmed by nucleotide sequencing of the cDNA (Chirala et al., 1989). Comparison of amino acid sequences of chicken synthase ACP to those of *E. coli* ACP and rat synthase ACP shows 23% and 65% homology, respectively. However, the sequence similarities are particularly remarkable around the seryl residue where the phosphopantetheine is attached. The high degree of homology in this region among the ACP domains of animal, plant, and bacterial synthases suggests the conserved nature of these sequences and reinforces the central functional role of ACP in fatty acid synthesis.

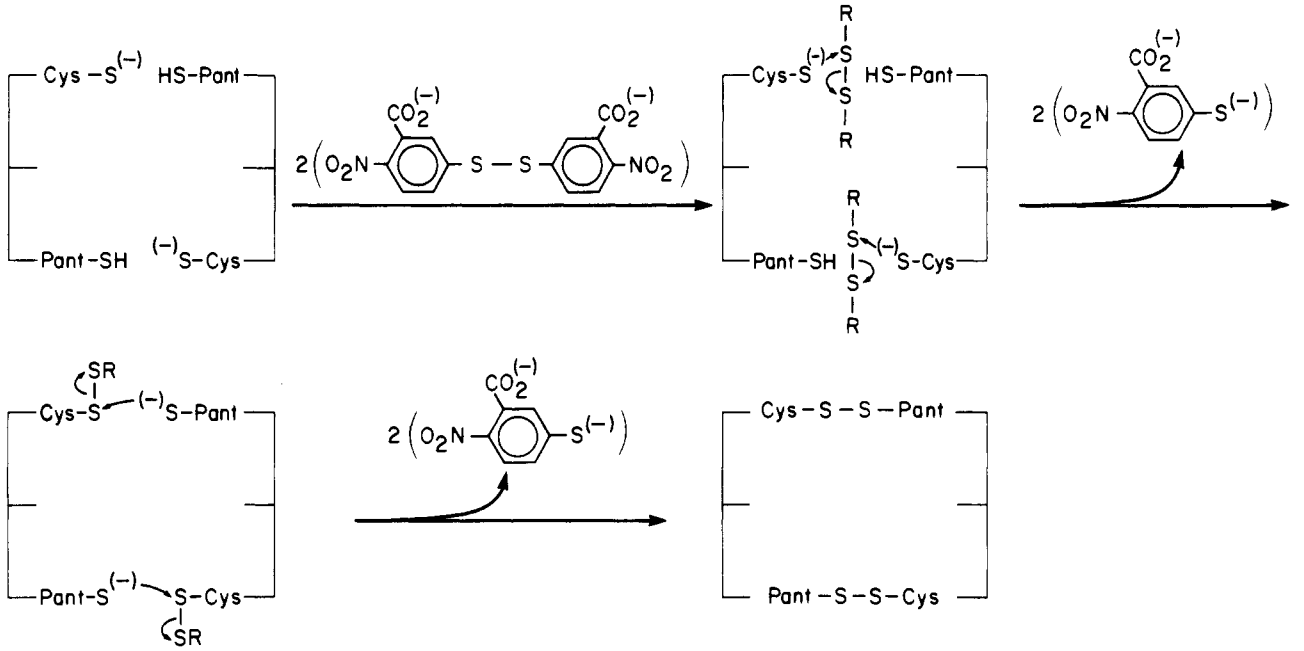
The nucleotide sequence of the chicken synthase cDNA shows that the ACP domain connects directly to the thioesterase domain through a stretch of 15 amino acid residues which does not appear to be conserved when compared to the rat synthase. The seryl residue to which the phosphopantetheine is attached is only 141 amino acids upstream from the active serine–OH of the thioesterase (Chirala et al., 1989), indicating that the two domains are highly compacted. The close proximity of the two active sites may be essential in facilitating the scanning of the growing fatty acyl chain by the thioesterase prior to its hydrolysis. Also, it may explain why the regeneration of synthase activity after chymotryptic cleavage of the thioesterase could not be reconstituted by addition of the thioesterase.

Recently, we have expressed the cDNA coding for the ACP and thioesterase domains in *E. coli* using a PL-promoter-based expression vector (unpublished results). The recombinant protein purified from *E. coli* extracts has thioesterase activity, and the ACP domain has 4′-phosphopantetheine attached to it by the *E. coli* ACP-synthetase (Elovson & Vagelos, 1968). The recognition, therefore, of the apo-ACP by the ACP–synthetase suggests that the ACP domain of the recombinant protein has a similar secondary structure as that of *E. coli* ACP and is a substrate for the synthetase, even though its amino acid sequence homology is only 23% (Huang et al., 1989). Similarly, the ACP–thioesterase recombinant protein appears to behave structurally and functionally equivalently to that of the native synthase domains. Thus, the folding of each of the domains appears to be an inherent property of the domain's primary sequence and is independent of the rest of the domains associated with the subunit polypeptide. This observation suggests that the functional domains of the multifunctional fatty acid synthase are folded independently and probably have arisen from fusion of genes coding for separate activities.

The native fatty acid synthase isolated from animal tissues is a dimer and is active in palmitate synthesis. Moreover, active enzyme centrifugation studies showed that the dimer is the only active species involved in fatty acid synthesis (Stoops et al., 1979). Dissociation of the native enzyme to monomers resulted in the loss of palmitate synthesis. However, six of the component enzymes remain active, including the acetyl and malonyl transacylases, β-ketoacyl and enoyl reductases, β-hydroxyacyl dehydratase, and thioesterase (Butterworth et al., 1967; Yung & Hsu, 1972; Muesing et al., 1975; Stoops et al., 1979; Stoops & Wakil, 1981). The one activity lost from the monomer is the β-ketoacyl synthase (condensing enzyme), yet its active cysteine–SH is present on the subunit (Stoops & Wakil, 1981). This result was puzzling since each subunit contains the domains of all component activities.

Earlier studies suggested that the acetyl and malonyl groups are bound to the synthase via thioester linkages (Joshi et al., 1970; Philips et al., 1970; Stoops & Wakil, 1982; Mikkelsen et al., 1985b). The acetyl group is bound to the cysteine–SH of the β-ketoacyl synthase and to the cysteamine–SH of the 4′-phosphopantetheine. The malonyl group, on the other hand, is bound only to the cysteamine–SH (Joshi et al., 1970; Plate et al., 1970; Philips et al., 1970; Stoops & Wakil, 1981, 1982). The cysteine–SH is unusually reactive at neutral pH or lower (Stoops & Wakil, 1981), indicating that this thiol most likely exists as a thiolate ion and therefore is highly susceptible to alkylation. The pantetheine–SH is also susceptible to alkylation and is specifically alkylated by chloroacetyl-CoA (Kumar et al., 1980; McCarthy & Hardie, 1982). The latter compound and reagents such as iodoacetamide, 1,3-dibromopropanone, or Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid) (DTNB)] alkylate the fatty acid synthase, resulting in the inhibition of palmitate synthesis. For instance, chicken liver synthase in the presence of 0.5 mM iodoacetamide is completely inhibited with a rate constant of 0.033 min^{-1} and $t_{1/2}$ of 21 min. The reaction follows first-order kinetics for over 90% inhibition of palmitate synthesis and is therefore consistent with the assumption that the inhibition resulted from the reaction of iodoacetamide with one catalytic site on the enzyme. When [^{14}C]iodoacetamide is used, over 80% of the ^{14}C label is recovered as *S*-([^{14}C]carboxymethyl)cysteine after HCl hydrolysis, indicating that the inhibition of the enzyme is due

Scheme I: Proposed Reactions of DTNB with Chicken Liver Fatty Acid Synthase Homodimer[a]



[a] Cys-S⁻ is cysteinyl-S⁻ and Pant-SH is pantetheine-SH.

to alkylation of an active cysteine-SH. This thiol is the active cysteine-SH of the β-ketoacyl synthase component of the fatty acid synthase since it is the only partial activity lost among the seven partial activities associated with the synthase (Stoops & Wakil, 1981). Preincubation of synthase with acetyl-CoA prior to its reaction with iodoacetamide protects the enzyme against inhibition, while preincubation with malonyl-CoA does not render such protection. These observations strongly indicated that alkylating reagents compete with the acetyl group for the same cysteine-SH at the β-ketoacyl synthase site.

In contrast, the reagents 1,3-dibromopropanone and DTNB react very rapidly (within 30 s) with the fatty acid synthase and inhibit palmitate synthesis almost completely (Stoops & Wakil, 1981, 1982). Survey of the partial activities associated with the inhibited enzyme showed that the loss of palmitate synthesis was due to loss of the β-ketoacyl synthase activity. All other partial activities remain intact after reaction of the fatty acid synthase with the alkylating reagents. Scatchard analysis of the inhibition of synthase by dibromopropanone or DTNB indicated that the binding of about 2 mol of the inhibitor/mol of dimer was required for the complete loss of palmitate synthesis.

When the dibromopropanone-inhibited synthase is analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, the synthase subunit of 260 kDa is nearly absent and new protein bands of doubled molecular weight (500 000–550 000) are found. If [^{14}C]dibromopropanone is used, nearly all the radioactivity is associated with the oligomers of higher molecular weight, indicating that synthase subunits are cross-linked by the bifunctional reagent, dibromopropanone. Preincubation of synthase with acetyl-CoA or malonyl-CoA prior to its reaction with dibromopropanone prevents the cross-linking of the subunits. Similarly, pretreatment of the enzyme with iodoacetamide prior to its reaction with dibromopropanone prevents cross-linking of the synthase subunits. These observations suggest that dibromopropanone cross-linking of the subunit takes place between the cysteine-SH (the site of acetyl binding or iodoacetamide reaction) and the cysteamine-SH of the prosthetic group, 4′-phosphopantetheine (the site of malonyl binding).

Further support for this organization is based on studies of the residues involved in cross-linking synthase subunits by dibromopropanone. When the fatty acid synthase was inhibited with [^{14}C]dibromopropanone and the cross-linked enzyme was subjected to performic acid oxidation [Baeyer–Villeger reaction (Hassal, 1957)] followed by HCl hydrolysis (Crestfield et al., 1963), equal amounts of ^{14}C-labeled sulfones of (carboxymethyl)cysteine and (carboxymethyl)cysteamine were obtained, indicating that the residues involved in the cross-linking are cysteine-SH and pantetheine-SH of the prosthetic group. This conclusion was supported further by the isolation of secondary propyl thioether derivatives of cysteine and cysteamine as the major products after reduction of the [^{14}C]dibromopropanone-cross-linked synthase with borohydride followed by HCl hydrolysis (Stoops & Wakil, 1983).

The subunits of DTNB-inhibited synthase are also cross-linked as shown by analysis with sodium dodecyl sulfate–polyacrylamide gel electrophoresis in an inert atmosphere (under Ar or N_2) and in the absence of 2-mercaptoethanol (Stoops & Wakil, 1982; Tian et al., 1985). When the DTNB-inhibited synthase is treated with 2-mercaptoethanol, the cross-linking is reversed with a concomitant regeneration of palmitate synthesis activity (Stoops & Wakil, 1982). Altogether, these observations indicate that the DTNB inhibition of the synthase results in formation of disulfide bridges between the active cysteine-SH and the cysteamine-SH of the two subunits as shown in Scheme I. Detailed kinetics studies of the reactivity of the synthase with DTNB and the effect of pH, salt concentration, and $NADP^+$ and NADPH on this reaction were reported by Tian et al. (1985).

Scheme I presupposes a head-to-tail organization of the two synthase subunits and the juxtaposition of their thiols within 2 Å of each other. This is a remarkable arrangement of the two subunit proteins considering their unusually large sizes.

Further support for this arrangement was obtained by hybridization experiments in which the phosphopantetheine-SH group was chemically modified by chloroacetyl-CoA and the cysteine-SH group by iodoacetamide (Wang et al., 1984). The resulting enzyme variants were dissociated and hybridized.

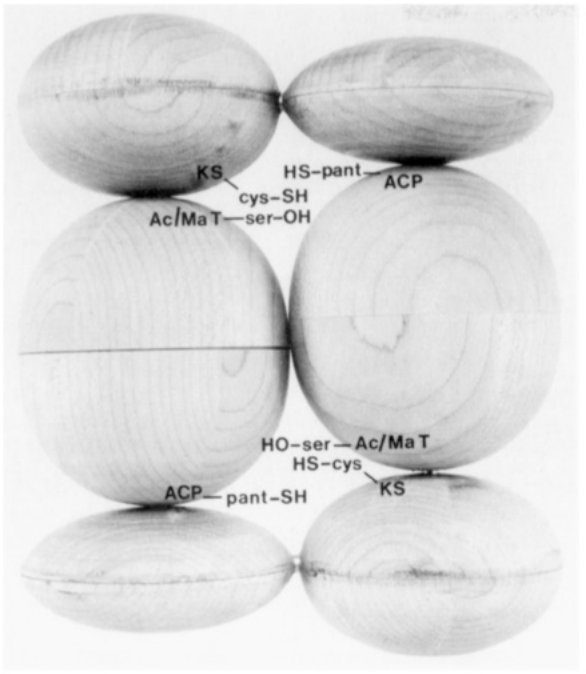


FIGURE 2: Model of chicken liver fatty acid synthase based on small-angle neutron-scattering studies. The model is two side-by-side cylinders with dimensions of 160 × 146 × 73 Å. Each cylinder has three domains measuring, respectively, 32, 82, and 62 Å in length and 36.5 Å in radius, and the domains are related by the dyad axis. Probable locations of the β-ketoacyl synthase and its active cysteine–SH (KS–cys–SH), the acetyl and malonyl transacylases and their common serine–OH (Ac/MaT–ser–OH), and the acyl carrier protein and its 4′-phosphopantetheine prosthetic group (ACP–pant–SH) are shown in the two crevices generated.

The hybrid containing an intact phosphopantetheine–SH group on one subunit and a cysteine–SH group on the other subunit was active in palmitate synthesis. Altogether, the aforementioned results strongly support the conclusion that the two identical subunits of animal synthases are organized in a head-to-tail arrangement so that the active cysteine–SH of one subunit is juxtapositioned opposite a pantetheine–SH of the adjacent subunit, generating two identical centers (Figure 1). Since the cysteine–SH is located at the active site of the β-ketoacyl synthase, the two centers obtained by this arrangement constitute novel β-ketoacyl synthase (condensing) sites. The acetyl group is attached to the cysteine–SH site, and the malonyl group is bound to the pantetheine–SH and therefore makes it possible for the condensation to occur yielding the β-ketoacyl-*S*-pantethenyl derivative.

This conclusion is supported further by physical studies of the synthase using electron microscopy and small-angle neutron-scattering techniques (Stoops et al., 1987). On the basis of these studies a structural model was proposed with an overall appearance of two side-by-side cylinders with dimensions of 160 × 146 × 73 Å (Figure 2). Each cylinder (160 Å in length and 36.5 Å in diameter) is divided into three domains having lengths of 32, 82, and 46 Å, respectively. In the antiparallel arrangement of the two cylinders, two crevices are generated on the major axis of the model at opposite ends of the molecular dyad (Figure 2). It is probable that the β-ketoacyl synthase is located in these crevices with its cysteine and pantetheine residues derived from adjacent subunits.

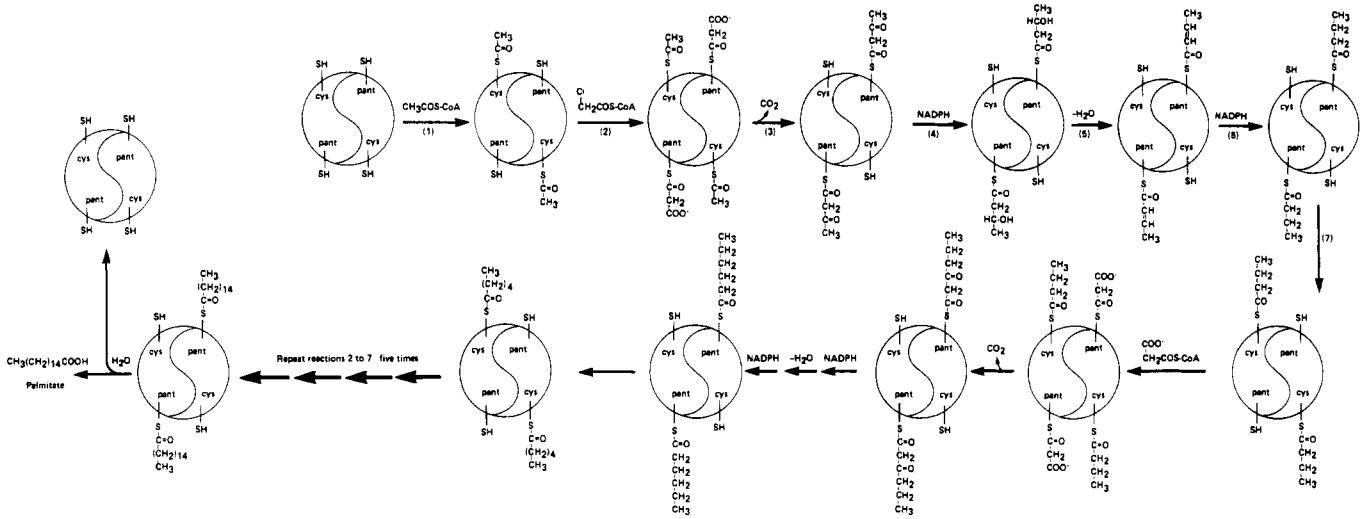
Since the dimeric complex possesses two complements of each catalytic activity involved in fatty acid synthesis, the model presented above predicts the presence of two active centers for fatty acid synthesis (Figures 1 and 2). Evidence in support of this prediction was recently obtained from chicken liver fatty acid synthase with its thioesterase activity either cleaved by chymotrypsin or inhibited by phenylmethanesulfonyl fluoride or diisopropyl fluorophosphate, thus ensuring that the fatty acyl products remain bound to the protein (Singh et al., 1984). By use of stoichiometric amounts of these enzyme preparations, it was possible to determine the kinetics and the amounts of NADPH oxidized and long-chain fatty acids synthesized. The results show that the amounts of NADPH consumed were sufficient to account for all the fatty acid synthesized and that 1.0 mol of fatty acyl enzyme is synthesized per mole of phosphopantetheine available, indicating that two sites for fatty acid synthesis are active and function simultaneously (Figure 1). The fatty acid synthase has, therefore, full-site reactivity and not half-site reactivity as was claimed earlier (Clements & Barden, 1979; Libertini & Smith, 1979).

Analysis of the fatty acyl products obtained with modified synthase that does not have thioesterase activity showed that $C_{20:0}$ and $C_{22:0}$ constituted over 85% of the fatty acids produced (Libertini & Smith, 1979; Singh et al., 1984). This is in contrast to the native enzyme, which yields mainly palmitate (70%) with stearate (20%) and myristate (10%) (Bressler & Wakil, 1961). These results indicate that the thioesterase is responsible for the chain termination of the process of fatty acid synthesis, since synthase is capable of chain elongating the bound fatty acyl group beyond the $C_{16:0}$ acid. However, in the presence of active thioesterase, the acyl group is preferentially hydrolyzed as it attains the $C_{16:0}$ chain length. This action reflects the specificity of the thioesterase, which has most of its activity on the palmitoyl-CoA and to a lesser extent on the stearoyl- and myristoyl-CoA derivatives (Lin & Smith, 1978; Crisp & Wakil, 1982).

The two centers of palmitate synthesis resulting from the antiparallel arrangement of the two identical subunits of synthase derive the required component activities from complementing halves of the two subunits. As depicted in Figure 1, one subunit contributes domain I while the second subunit contributes domains II and III. This novel organization of these proteins is not surprising since the reactions leading to palmitate synthesis are complex and the component activities are distributed all along the lengthy polypeptide chain, thereby making their interaction difficult if the active synthase is a monomer.

Domain I catalyzes the entry of the acetyl and malonyl substrates into the process of palmitate synthesis. These entries are catalyzed by the acetyl and malonyl transacylases. The acetyl transacylase is specific for acetyl-CoA, but can to a lesser degree utilize propionyl- and butyryl-CoAs as primers in fatty acid synthesis. Malonyl transacylase, on the other hand, is specific for malonyl-CoA. Both transacylases transfer the acyl groups from CoA to an active serine–OH residue on the protein. In the animal synthases the acetyl and malonyl groups competitively inhibit each other's binding to the protein, suggesting that they bind to a common active site (Joshi et al., 1970; Plate et al., 1970; Stern et al., 1982; McCarthy & Hardie, 1983). This was confirmed by the isolation and sequencing of the peptides derived from acetyl and malonyl synthases (McCarthy et al., 1983; Mikkelsen et al., 1985a). Both the acetyl and malonyl groups are bound as an O-ester prior to their transfer to appropriate thiols. The sequence of the amino acids of the peptide containing this serine is highly conserved and retains the sequence motif of -Gly-X-Ser-Y-Gly- that characterizes enzymes with a reactive serine, such as yeast acetyl and malonyl/palmitoyl transacylases, synthase thioesterases, human plasmin, and bovine trypsin and carboxyl

Scheme II: Diagrammatic Scheme of Fatty Acid Synthesis by Full-Sites-Active Chicken Liver Fatty Acid Synthase



esterase (Barker & Dayhoff, 1972; Hardie & McCarthy, 1986; Yang et al., 1988).

The binding of the proper substrate (acetyl or malonyl) to the enzyme serine–OH is critical in ensuring proper entry of substrates into fatty acid synthesis. To facilitate this process, free CoA–SH plays an important role in the binding of the correct substrate, an acetyl or malonyl group (Lin et al., 1980; Stern et al., 1982; Soulie et al., 1984). Removal of CoA–SH by a scavanging reaction such as that of citrate lyase stops fatty acid synthesis. If the wrong substrate is bound, CoA–SH acts as an acceptor and removes the acyl group, thereby regenerating the active serine–OH for binding of the proper acyl group needed at that particular moment in the repetitive process (Figure 3). If a primer is needed, an acetyl group is bound, and if a source of C_2 units for chain elongation is needed, a malonyl group is bound.

The next step in the entry of the substrates is the channeling of the acetyl and malonyl groups to their proper thiols. The malonyl group binds only to the cysteamine–SH of the prosthetic group, 4′-phosphopantetheine; hence, it is presumed to be channeled directly from the active serine O-ester to the ACP–pantetheine–SH (Figure 3) (Joshi et al., 1970; Phillips et al., 1970). The acetyl group, on the other hand, binds to both the active cysteine–SH of the β-ketoacyl synthase and to the cysteamine–SH of ACP (Joshi et al., 1970; Mikkelsen et al., 1985b). It is not clear, therefore, whether the acetyl group is transferred directly from the serine O-ester to the cysteine–SH or whether it is first transferred to the cysteamine–SH and then to the cysteine–SH of the condensing domain. In the fatty acid synthesizing system of bacteria, the answer is clear because both acetyl–S–ACP and malonyl–S–ACP are required for the β-ketoacyl synthase reaction. The acetyl group is transferred from the ACP to the active cysteine–SH of the condensing enzyme prior to its condensation with the malonyl group to form acetoacetyl–S–ACP (reactions 4 and 5). In the animal fatty acid synthase it has not been possible as yet to determine which of the two pathways the acetyl group follows.

Recently, the acetylation of the yeast fatty acid synthase was studied in detail with specific alkylating reagents for the cysteine–SH and cysteamine–SH (N. Singh, J. K. Stoops, and S. J. Wakil, unpublished data). For instance, iodoacetamide specifically alkylates the cysteine–SH, chloroacetyl-CoA alkylates the cysteamine–SH, and dibromopropanone alkylates both thiols. The kinetics and stoichiometry of acylation of the alkylated yeast synthase were then studied with *p*-nitrophenyl

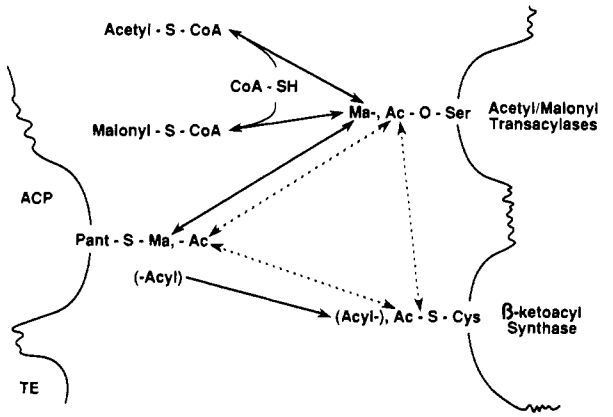


FIGURE 3: Role of CoA–SH in the self-editing of the loading of acetyl (Ac–) and malonyl (Ma–) groups to the common active Ser–OH of the acetyl and malonyl transacylases of animal fatty acid synthase. During each of the eight loading steps the enzyme site is partitioned between competently and incompetently bound substrate molecules. The transfer of the malonyl group from the Ser–OH to the pantetheine–SH (Pant–S–) and of the acyl product of each elongation step (Acyl–) from Pant–S– to the ketoacyl synthase–Cys–SH is shown by solid arrows denoting their more likely route. The transfer of the Ac from Ser–O to Pant–S to Cys–S is represented by dotted arrows denoting the uncertainty in the involvement of the Pant–SH in the Ac loading of Cys–SH of the ketoacyl synthase.

thioacetate. The results show that acetylations of the seryl–OH and cysteine–SH are fast, while acetylation of the cysteamine–SH is very slow and could be discounted. In the yeast synthase at least, the pathway of acetyl binding to the enzyme proceeds from CoA to the serine–OH of the acetyl transacetylase to the cysteine–SH of the β-ketoacyl synthase. No such studies have been carried out with the animal synthase because of technical difficulties. However, Yuan and Hammes (1985) have investigated this problem in chicken liver synthase and have concluded that the acetyl moiety is transferred to the cysteine–SH from the cysteamine–SH, a proposal parallel to reaction 5 (i.e., all sites are acetylated from CoA). Their kinetic data, however, could be explained equally well by direct transfer of the acetyl group from serine–OH to the cysteine–SH.

On the basis of these observations the following mechanism for palmitate synthesis has been proposed (Scheme II). The active synthase is charged with an acetyl group at the cysteine–SH of the β-ketoacyl synthase and with the malonyl group at the cysteamine–SH of the adjacent subunit. Condensation takes place by coupling the carbonyl group of the acetyl moiety

to the β-carbon of the malonyl group with a simultaneous release of CO_2 and formation of an acetoacetyl product. The cysteine–SH of the β-ketoacyl synthase is reset to the free thiol state. The acetoacetyl-*S*-pantethenyl derivative is then reduced with NADPH (β-ketoacyl reductase; reaction 6) to the β-hydroxybutyryl derivative, which is then dehydrated by the dehydratase (reaction 7) and reduced by NADPH (enoyl reductase; reaction 8) to the butyryl derivative. All these enzymes are present within domain II and accessible to the pantetheine prosthetic group at the ACP domain. The butyryl product is then transferred to the cysteine–SH of the condensing enzyme in domain I of the adjacent subunit, thereby freeing the cysteamine–SH to accept a malonyl group from malonyl-CoA. The butyryl and malonyl groups condense to form a β-ketohexanoyl-*S*-pantethenyl intermediate which is then reduced to a hexanoyl derivative. The process is then repeated five more times; with each cycle the acyl group is elongated by a C_2 unit, ultimately yielding a palmitoyl derivative. The latter is then hydrolyzed by the neighboring thioesterase to form palmitic acid and the cysteamine–SH. The essence of this mechanism is (1) the organization of the two homodimers of the synthase so that the binding sites of the primer and the elongating malonyl groups are within bond distances, (2) the involvement of complementary domains derived from the two subunits in the condensation reaction and the transfer of the acyl group between the cysteine–SH and pantetheine–SH of the two subunits with each cycle adding C_2 units, and (3) the presence of two active centers for the synthesis of palmitate within the synthase dimer, with each independent center having its own complement of enzymes. The multifunctional nature of the subunits and their head-to-tail organization produce a highly efficient enzyme complex capable of carrying out multiple reactions leading to the synthesis of a palmitate molecule from one acetyl and seven malonyl moieties. Moreover, the entire system is designed so that intermediate acyl derivatives remain efficiently anchored to the enzyme during conversion to the product acid. With the exception of some highly specialized tissues (e.g., lactating mammary gland), the useless shorter chain byproducts (C_6 through C_{12}) are not produced. This remarkable multifunctional enzyme system has evolved into a proficient machine that carries out the synthesis of long-chain fatty acids from simple building blocks of C_2 units.

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