insulating substrates by measurement of the feedback current really represent two limiting cases where the heterogeneous electron transfer (et) to tip-generated species is either very fast or very slow. However, measurements at finite et rates at the substrate are also possible and lead to steady-state i_T vs d curves that span the region between the limiting cases. Similarly it should be possible to measure the porosity of a nonconductive film coating a conductive substrate. If the tip radius, a, is small compared to the pore size, the actual pore distribution can be determined. Even for pores that are small compared to a, information about the porosity should be obtainable from the i_T vs d curve. SECM studies of polymer films or other types of modified electrode surfaces have been described earlier. It should also be possible to modify the tip electrode, e.g., with an organized monolayer or polymer film, and study et reactions between it and a suitable substrate. Such studies would be related to those carried out in the ultrathin-layer cell[27] but, with proper tip electrode construction, may be somewhat easier to execute.

While most SECM studies involved steady-state currents and studies of substrate properties, measurements of current transients should also be informative, e.g., in determination of diffusion coefficients and rate constants. Measurement of steady-state and transient currents might also be used to characterize homogeneous reactions of the tip-generated species that occur in the solution gap between the tip and substrate, in a manner analogous to measurements made with the rotating ring-disk electrode.[10,28] These applications await the development of suitable models, probably digital simulation ones.

Finally, many applications to the characterization and modification of insulating and conducting substrates are possible. The ultimate resolution possible with the SECM depends upon our ability to construct scanning tips of a small size of the proper shape. While it is unlikely that the resolution will ever approach that of the STM, improvements to bring SECM to the levels of several tens of nanometers, by reduction in tip size and by employing deconvolution or tomographic-type techniques, should be possible.

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Structure and Mechanism of Action of a Multifunctional Enzyme: Fatty Acid Synthase

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The synthesis of fatty acids is a ubiquitous process in nature. However, a variety of different enzymes catalyze the synthesis, and their structures display considerable variation. In this account, we will be concerned with the fatty acid synthase from chicken liver which primarily produces palmitic acid. In most procaryotes, the synthesis of palmitic acid is carried out by seven different and separable monofunctional enzymes and an acyl carrier protein (cf. refs 1 and 2). However, in eucaryotes a multifunctional enzyme is involved that contains the seven enzyme activities. In yeast and other fungi, a multifunctional enzyme of two different types of polypeptides is found ($\alpha_6\beta_6$),[3] whereas in animals, the enzyme consists of two identical polypeptides (α_2).[1,2] The multifunctional enzymes in animals catalyze a reaction sequence that leads to palmitic acid according to the overall reaction

$$\text{acetyl-CoA} + 7\text{malonyl-CoA} + 14\text{NADPH} + 14\text{H}^+ \rightarrow \text{palmitic acid} + 8\text{CoA} + 14\text{NADP}^+ + 6\text{H}_2\text{O} + 7\text{CO}_2 \quad (1)$$

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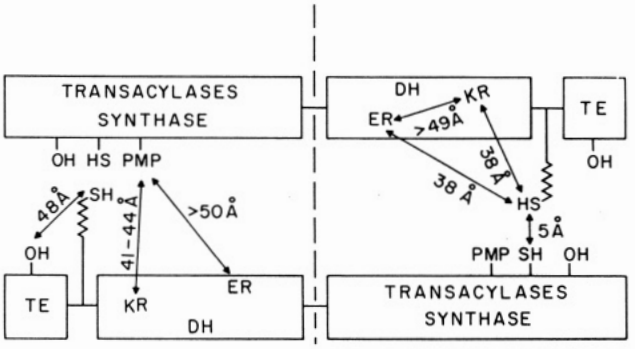

Figure 1. Schematic representation of the fatty acid synthase structure. The two polypeptide chains and the binding sites that have been labeled with fluorescent/absorbance probes are shown, as well as some of the measured distances. The dashed line separates the two independent catalytic centers, each composed of two different polypeptide chains. DH is dehydratase; ER is enoyl reductase; KR is β-ketoacyl reductase; TE is thioesterase; PMP is pyridoxamine phosphate (site I); and ~SH is 4′-phosphopantetheine. The essential cysteine (–SH) and serine (–OH) are shown.[10]

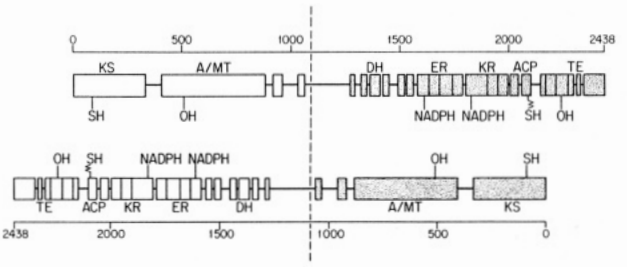

Figure 2. Functional map of chicken liver fatty acid synthase. Boxes are regions of great homology with the rat enzyme, whereas the lines identify less homologous regions and are often connections between structural domains. The essential cysteine (–SH), serine (–OH), 4′-phosphopantetheine (~SH), and NADPH binding sites are shown. KS is β-ketoacyl synthase; A/MT is acetyl/malonyl transacylase; DH is dehydratase; ER is enoyl reductase; KR is β-ketoacyl reductase; ACP is acyl carrier protein; and TE is thioesterase. The numbering of the amino acids also is given.[24] The rat polypeptide has 74 additional amino acids on the N-terminus.[20] If these amino acids also are present on the chicken liver polypeptide, the numbering of amino acids will be changed.

Multifunctional enzymes have several potential physiological advantages, for example, enhancement of the intrinsic catalytic activity of individual enzymes, sequestering of reaction intermediates, and regulation of metabolic fluxes.

Palmitic acid is generated by initiating the fatty acid chain with the acetyl group from acetyl-CoA and adding two carbons at a time from seven malonyl-CoA molecules. The growing fatty acid chain is covalently bound to the enzyme: during the catalytic reactions, it is attached through a thioester linkage to the sulfhydryl group of 4′-phosphopantethoic acid which is covalently linked to a serine residue of the protein. The specific sequence of reactions is as follows:

$$\text{acetyl-CoA} + \text{E} \rightleftharpoons \text{acetyl-E} + \text{CoA} \quad (2)$$

$$\text{malonyl-CoA} + \text{acetyl-E} \rightleftharpoons \text{acetyl-E-malonyl} + \text{CoA} \quad (3)$$

$$\text{acetyl-E-malonyl} \rightleftharpoons \text{CH}_3\text{C(O)CH}_2\text{C(O)-E} + \text{CO}_2 \quad (4)$$

$$\text{CH}_3\text{C(O)CH}_2\text{C(O)-E} + \text{NADPH} + \text{H}^+ \rightleftharpoons \text{CH}_3\text{CH(OH)CH}_2\text{C(O)-E} + \text{NADP}^+ \quad (5)$$

$$\text{CH}_3\text{CH(OH)CH}_2\text{C(O)-E} \rightleftharpoons \text{CH}_3\text{CH=CHC(O)-E} + \text{H}_2\text{O} \quad (6)$$

$$\text{CH}_3\text{CH=CHC(O)-E} + \text{NADPH} + \text{H}^+ \rightleftharpoons \text{CH}_3\text{CH}_2\text{CH}_2\text{C(O)-E} + \text{NADP}^+ \quad (7)$$

The sequence of enzyme activities is as follows: acetyl transacylase, malonyl transacylase, β-ketoacyl synthase, β-ketoacyl reductase, dehydratase, and enoyl reductase. In the chicken liver enzyme, the acetyl and malonyl transacylase activities are combined in a single enzyme.[4] When the fatty acid chain of the intermediate is saturated (eq 7), a malonyl residue is transferred to the enzyme (eq 3) and two more carbons are added. This cycling continues until a palmitoyl thioester is formed, which is cleaved by a palmitoyl thioesterase to give the free acid.

Structure of Fatty Acid Synthase

Chicken liver fatty acid synthase has a molecular weight of about 534 000 and consists of two identical polypeptide chains (cf. refs 1, 2, and 5). These polypeptides are arranged head to tail and combine to give two independent catalytic centers,[6] each derived from two different polypeptides. This is illustrated in Figure 1 where the two independent catalytic centers are separated by a vertical dashed line. This structure was deduced from many different lines of evidence. For example, if the two polypeptide chains are separated, all of the catalytic activities are intact except for the β-ketoacyl synthase and the β-ketoacyl reductase.[1,7] Also, the cysteine on the β-ketoacyl synthase of one polypeptide chain has been cross-linked to the 4′-phosphopantetheine on a different polypeptide chain with 1,3-dibromo-2-propanone,[8] indicating that these two sulfhydryls are within 5 Å of each other.

The cDNA coding for the polypeptide has been sequenced and the corresponding amino acid sequence deduced.[5,9] The active-site regions of the component enzymes, which are indicated in Figures 1 and 2, were primarily identified from amino acid sequencing of active site labeled peptides.[4,10] Starting from the C-terminus, the enzymes are thioesterase, β-ketoacyl reductase, enoyl reductase, dehydratase, acetyl/malonyl transacylase, and β-ketoacyl synthase. The growing fatty acid chain is bound to 4′-phosphopantetheine in a region between the thioesterase and β-ketoacyl reductase; this region is often called the "acyl carrier protein" because an isolatable protein carrying out this function is found in *Escherichia coli*. This sequence of activities also has been deduced from studies of limited proteolytic digestion of the multifunctional enzyme.[1,2,11]

The three-dimensional structure of the enzyme remains to be elucidated. However, small-angle neutron scattering and electron microscope studies suggest that

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the polypeptides have dimensions of 160 × 146 × 73 Å with three domains, 32, 82, and 46 Å in length.[12] In addition, fluorescence resonance energy transfer and electron paramagnetic resonance have been used to determine the distances between specific sites on the enzyme.[10,13,14]

Because of the size of the protein, specific chemical labeling is difficult. However, a number of fluorescent and ultraviolet–visible absorbing probes that label specific sites were developed and their locations determined by protein sequencing. The sites labeled are as follows: 4′-phosphopantetheine, serine at the active site of the thioesterase, lysine at the active site of enoyl reductase, and a specific lysine (site I) located in a domain on the N-terminus half of the polypeptide.[10,13] In addition, a variety of analogues of NADPH, including spin labels, were utilized.[10,14] The distances determined between specific sites and the locations of the sites within the structural model are included in Figure 1. The 4′-phosphopantetheine is about 20 Å long so that if the growing chain is to reach all of the catalytic sites, they must lie within a circle 40 Å in diameter. Some of the measured distances exceed this dimension; although the uncertainty is sufficient to prevent a firm conclusion, relatively large conformational changes may be part of the catalytic cycle. (The uncertainty in the distances due to experimental and theoretical factors is about 10–20%. However, the fluorescence probes are rather large so that the precise location of the transition dipole relative to the specific amino acid residue is unknown. This can lead to uncertainties as large as 10–20 Å in locating the specific sites.)

The thioesterase domain is at the C-terminus and can be readily cleaved from the enzyme by proteolysis.[1,2,15] If the thioesterase is cleaved from the native enzyme with trypsin, it cannot interact with the rest of the enzyme to hydrolyze the long-chain fatty acyl thioester. The "clipped" enzyme still performs all the catalytic reactions necessary to synthesize a long-chain fatty acyl thioester. Dynamic fluorescence anisotropy measurements of the enzyme, with the thioesterase active site serine specifically labeled with a fluorescent probe, indicate a rotational correlation time of the intact thioesterase of 44 ns.[13] This is not very different from the rotational correlation time of the trypsin fragment containing the thioesterase, 17 ns, but is much shorter than that for the entire enzyme, 610 ns.[16] This indicates that the thioesterase has considerable freedom of motion and therefore is capable of large conformational changes within the multifunctional enzyme during catalysis. Such a conformational change might be important during catalysis since the thioesterase site is relatively far from 4′-phosphopantetheine (Figure 1).

Evolution of Fatty Acid Synthase

The evolutionary aspects of fatty acid synthase are of interest in determining how multifunctional enzymes were developed from individual enzymes. The strong similarities between several substrate binding sites from different species suggest that the multifunctional polypeptides from yeast and animals have evolved from the monofunctional enzymes of lower species.[4,17-19]

Recently, the sequencing of the full cDNA encoding the chicken liver,[5,9] rat,[20] and yeast fatty acid synthase[21-23] has been reported. To obtain further information about conserved functionally important domains and evolutionary relationships among the enzymes, a homology analysis of the protein sequences has been carried out.[24] The entire amino acid sequences of the chicken and rat enzymes are 67% identical. If conservative substitutions are allowed, 78% of the amino acids are matched. A region of low homologies exists between the functional domains. In particular, a string of 206 amino acids in a region of low homologies is located at amino acid residues 1059–1264 of the chicken enzyme. This region connects two functionally different parts of the polypeptide: that is, as shown in Figures 1 and 2, in forming a multienzyme reaction center for fatty acid synthesis, the β-ketoacyl synthase and acetyl/malonyl transacylase interact with a different polypeptide containing the other enzymes and acyl carrier.

The arrangement of the catalytic sites of chicken liver fatty acid synthase by homology analysis of the protein sequence with the rat enzyme is represented in Figure 2. Boxes and lines are regions of high and low homologies with the rat enzyme, respectively, as defined in ref 24. The connecting regions between the individual enzymes of the rat and chicken fatty acid synthase have relatively low homologies. This suggests either that during evolution different joining events occurred for the two species or that the rat and chicken synthase share a common ancestor with a high rate of mutation occurring in the joining regions. A higher rate of mutations in the joining sequences than in the active sites of the enzymes without loss of function would not be unexpected.

A high degree of homology exists between the active sites of the chicken and rat enzymes, supporting the hypothesis that structurally conserved enzymes were combined during the evolutionary process. A lower degree of homology exists between the active sites of the chicken and yeast enzymes, suggesting that either the yeast fatty acid synthase diverged from the evolutionary path of the rat and chicken enzymes at a very early time during the joining process or the joining process occurred on a different evolutionary pathway.

Mechanism of Action of Fatty Acid Synthase

During the catalytic cycle, the reaction intermediates are covalently attached to three different sites on the enzyme: the acetyl and malonyl residues are loaded

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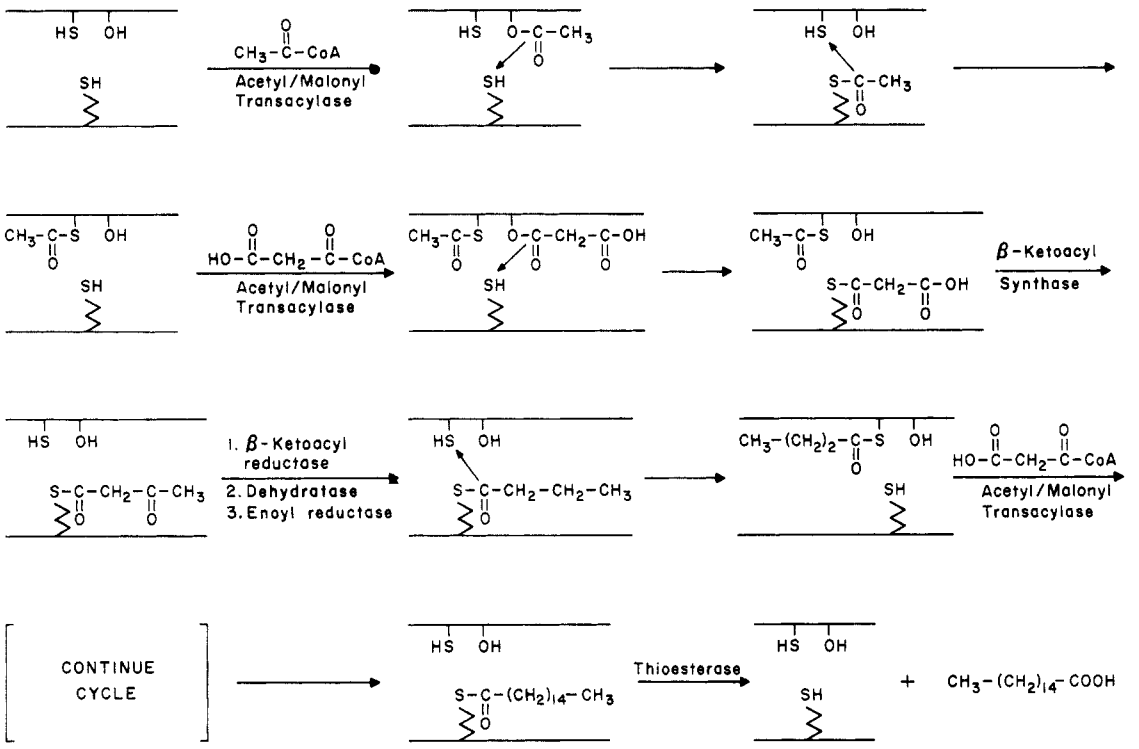

Figure 3. Schematic representation of palmitic acid synthesis by chicken liver fatty acid synthase. ~SH is 4′-phosphopantetheine, -SH is a cysteine sulfhydryl, and -OH is a serine hydroxyl. The initial sequence of reactions, malonyl transacylase, β-ketoacyl synthase, β-ketoacyl reductase, dehydratase, and enoyl reductase, is repeated seven times to give enzyme-bound palmitic acid.

onto the enzyme as an oxyester on serine and are then passed to 4′-phosphopantetheine, where a thioester linkage is formed; the condensation, reduction, dehydration, reduction (eqs 4–7), and thioesterase reactions occur with the malonyl and unsaturated growing fatty acid chain attached at this site. The saturated growing fatty acid chain is linked covalently to a cysteine sulfhydryl during the loading of the malonyl group and condenses with the malonyl group on 4′-phosphopantetheine. The reaction cycle and location of the bound intermediates are shown in Figure 3. The locations of the serine hydroxyl, 4′-phosphopantetheine, and cysteine on the enzyme are shown in Figures 1 and 2.

The evidence supporting this mechanism can be illustrated by studies of the binding of acetyl and malonyl to the enzyme after reaction with acetyl-CoA and malonyl-CoA. Early studies[25-28] demonstrated that acetyl binds to both 4′-phosphopantetheine and cysteine, whereas malonyl binds only to the former. If the acetylated enzyme is treated with hydroxylamine, all of the acetyl groups are rapidly removed.[29] However, if the acetylated enzyme is denatured and then treated with hydroxylamine, only about 60% of the acetyl groups are removed. Hydroxylamine cleaves thioesters but not normal oxyesters. These results indicate that acetyl is bound to the enzyme as thioesters and an unstable oxyester. Analyses of the binding of a fluorescent analogue of acetyl-CoA to the enzyme suggest two different types of sulfhydryl groups.[30,31] These results are consistent with the mechanism described above. The amino acid sequences of peptides associated with the serine, cysteine, and 4′-phosphopantetheine sites have been obtained.[4] These sites are indicated in Figure 2.

The location of reaction intermediates on the enzyme has been investigated by labeling the growing fatty acid chains on the enzyme with radioactivity.[32] If the thioesterase is cleaved from the enzyme prior to labeling, the labeled intermediates accumulate on the enzyme. After limited trypsinization of the enzyme, enzyme fragments containing either cysteine or 4′-phosphopantetheine can be isolated. The intermediates then can be released from the fragments and analyzed by high-performance liquid chromatography. The nature of the intermediates (e.g., extent of saturation, chain length) can be controlled by adjusting the substrate concentrations.

For example, if NADPH is present in limited amounts, predominantly 3-hydroxybutyryl-enzyme is formed, and >90% of the 3-hydroxybutyryl is found on 4′-phosphopantetheine. However, when the 3-hydroxybutyryl-enzyme is reduced with NADPH, the butyrate is found in approximately equal amounts on cysteine and 4′-phosphopantetheine. The unreduced species, acetoacetate, also is found primarily on 4′-phosphopantetheine. This is consistent with the transformation of acetoacetate to butyrate taking place with the intermediate on 4′-phosphopantetheine and butyrate then being transferred to cysteine.

The ratio of saturated intermediate bound on cysteine to that on 4′-phosphopantetheine has been determined as a function of the fatty acid chain length. The ratio is in the range 2–4 for C_{14} or less but drops to 0.3

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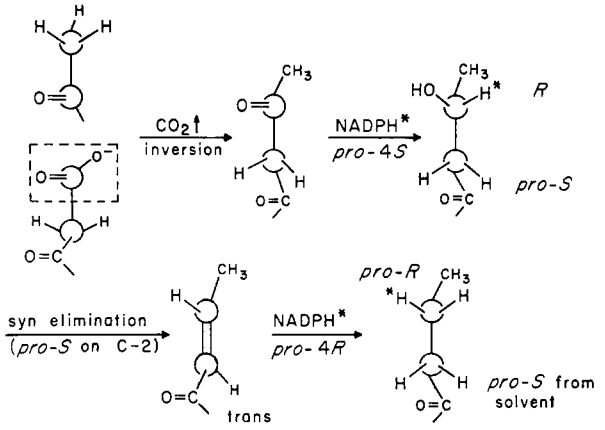

Figure 4. Stereochemistry of the reactions catalyzed by chicken liver fatty acid synthase as described in the text.[33]

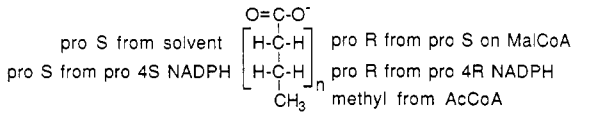

Figure 5. Origin of the prochiral and methyl hydrogens of fatty acids synthesized by chicken liver fatty acid synthase, as inferred from stereochemical studies of the synthesis of butyrate.[33]

for C_{16} and is undetectable for C_{18}. (Hydrolysis of palmitic acid does not occur because the thioesterase has been removed from the enzyme.) This suggests that the chain-length specificity of the enzyme is determined by the inability of chain lengths greater than C_{14} to be transferred to the cysteine. This could be due to steric restrictions on the intermediate binding site associated with the cysteine. The difficulty in chain elongation beyond C_{16} enhances the probability of termination at this chain length.

The mechanism in Figure 3 is consistent with all existing data. However, there is some indication from isotopic distribution studies that the initial acetyl bonding may not be completely ordered, that is, the acetate may react directly with the cysteine and 4′-phosphopantetheine, as well as with the serine hydroxyl.[32]

The stereochemistry of the sequence of reactions in Figure 3 has been elucidated as shown in Figure 4.[33] The condensation of malonyl and acetyl proceeds with inversion of C-2 of malonyl. The next step, the reduction of acetoacetyl-CoA to 3-hydroxybutyryl-CoA, occurs with the transfer of the *pro*-4*S* hydrogen of NADPH, a conserved feature of all fatty acid syntheses. The elimination of water is syn with the *pro-S* hydrogen from C-2 being removed. The reduction of crotonyl-CoA to butyryl-CoA utilizes the *pro*-4*R* hydrogen of NADPH which is transferred to the *pro*-3*R* position of butyryl-CoA, with the concomitant addition of a solvent proton to the *pro*-2*S* position of butyryl-CoA.

As shown in Figure 5, with the exception of the methyl hydrogens on the terminal carbon derived from the primer acetyl group, the hydrogens on the carbons of the fatty acid synthesized can be traced to a unique source. The *pro-R* and *pro-S* hydrogens on the even carbons are derived from the *pro-S* hydrogen of malonyl-CoA and from solvent, respectively, whereas the *pro-R* and *pro-S* hydrogens on the odd carbons are derived from the *pro*-4*R* and *pro*-4*S* hydrogens of NADPH, respectively. As an additional benefit, knowledge of the stereochemistry permits synthesis of specifically deuterated or tritiated palmitic acid.

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Kinetics of Fatty Acid Synthase

The kinetics of fatty acid synthesis are quite complex, as might be expected, and a variety of techniques must be used to obtain information about the elementary steps in the catalytic process. The steady-state kinetics follow a typical Michaelis–Menten rate law for the three substrates, acetyl-CoA, malonyl-CoA, and NADPH.[34] An unusual feature of the rate law is that malonyl-CoA is a competitive inhibitor of acetyl-CoA and acetyl-CoA is a competitive inhibitor of malonyl-CoA. The rate law is in accord with the mechanism in Figure 3, with acetyl-CoA and malonyl-CoA competing for the same "loading" sites on the enzyme.

The steady-state reaction is assayed by following the decrease in absorbance as NADPH is oxidized to $NADP^+$. If phosphotransacetylase is used to rapidly scavenge CoA from the assay mixture, the synthesis of fatty acid stops.[35,36] Although CoA is a product of the reaction, it is essential for catalysis. In fact, at low concentrations, CoA is an activator, and at high concentrations, it is an inhibitor. The explanation for this unusual result is that CoA rapidly exchanges acetyl and malonyl groups with the enzyme. This permits the rapid exchange of acetyl and malonyl groups between binding sites on the enzyme. Without CoA, for example, acetyl could form a thioester with 4′-phosphopantetheine which would dissociate from the enzyme very slowly, thereby preventing malonyl from forming a thioester with 4′-phosphopantetheine and subsequent synthesis of palmitic acid.

If the rates of the catalytic steps are assumed to be independent of the chain length of the fatty acid, the turnover number of the overall reaction, k_{cat}, can be written as

$$1/k_{cat} = (1/k_1 + 7/k_2 + 7/k_3 + 7/k_4 + 7/k_5 + 7/k_6 + 1/k_7) \quad (8)$$

where k_1, k_2, k_3, k_4, k_5, k_6, and k_7 are the turnover numbers for acetyl transacylase, malonyl transacylase, β-ketoacyl synthase, β-ketoacyl reductase, dehydratase, enoyl reductase, and thioesterase, respectively.

The overall reaction has been dissected into elementary steps through the use of fast-reaction techniques (cf. ref 37). The acetylation of fatty acid synthase by acetyl-CoA (Ac-CoA) has been studied with quenched-flow methods.[29] The kinetic data are consistent with a mechanism involving relatively rapid binding of Ac-CoA to the enzyme followed by acetylation:

$$\text{Ac-CoA} + \text{E} \rightleftharpoons \text{E·Ac-CoA} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{EAc·CoA} \rightleftharpoons \text{EAc} + \text{CoA} \quad (9)$$

At pH 7.0 and 23 °C, $k_1 = 43\ s^{-1}$ and $k_{-1} = 103\ s^{-1}$. The removal of acetyl from the enzyme by CoA is quite rapid, consistent with its effect on the steady-state

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kinetics. The above reaction characterizes the overall rate of acetylation of the enzyme; however, three different binding sites for acetyl exist on the enzyme. Information about the rate of acetylation of the individual binding sites can be obtained by specifically blocking either the cysteine or cysteine and 4′-phosphopantetheine through chemical modification of the protein.[38] As expected, formation of the oxyester with serine is relatively rapid, with $k_1 = 150\ s^{-1}$. If acetylation of the three binding sites is random, then for binding to 4′-phosphopantetheine, $k_1 < 110\ s^{-1}$; and for binding to cysteine, $k_1 < 43\ s^{-1}$. If the reaction is strictly ordered as in Figure 3, the rate constant for intramolecular transfer of acetyl from serine to 4′-phosphopantetheine is $<110\ s^{-1}$ and for transfer from 4′-phosphopantetheine to cysteine is $<43\ s^{-1}$.

The enzyme is quite specific for acetyl-CoA: acetoacetyl-CoA, crotonyl-CoA, and 3-hydroxybutyryl-CoA react much more slowly, making them relatively poor primers.[39] (This is true for avian enzymes but not for mammalian enzymes where butyryl-CoA, and even longer primers, are very effective.[40])

The kinetics of malonylation of the enzyme by malonyl-CoA has not been studied, primarily because of the high cost of radioactive substrate and the complex side reactions that occur.[41]

The binding of NADPH to the enzyme is accompanied by a large enhancement of the fluorescence of NADPH. The kinetics of binding is consistent with a simple bimolecular reaction, in spite of the fact that binding occurs at the active sites of two different reductases.[42] At pH 7.0, 25 °C, the second-order rate constant is approximately $10^7\ M^{-1}\ s^{-1}$. In fact, the structure of bound NADPH differs at the active sites of the two reductases: nuclear Overhauser effect measurements indicate that the conformation of the nicotinamide–ribose bond is anti at the enoyl reductase site and syn at the β-ketoacyl reductase site.[43] These structures are consistent with the stereochemistry of the proton transfer from NADPH discussed earlier.

To study the combination of acetyl and malonyl enzyme and the subsequent reduction of acetoacetyl to 3-hydroxybutyryl, conditions must be found such that the reaction is terminated when 3-hydroxybutyryl is formed and the kinetics are relatively simple. In addition, a method must be devised for following the course of the reactions. The binding of NADPH to the enzyme is sufficiently fast that the enhancement of NADPH fluorescence can be used as an indicator to follow the course of the reactions. If the concentrations of acetyl-CoA and malonyl-CoA are large relative to the enzyme concentration, the formation of acetoacetyl-enzyme is a first-order reaction (with respect to the enzyme). If this is coupled to the subsequent β-ketoacyl reductase reaction under conditions where the enzyme concentration is much greater than the NADPH concentration, the reduction is first order (with respect to NADPH), and the reaction stops when 3-hydroxybutyryl is formed because insufficient NADPH is present for the synthesis of palmitic acid to proceed further. The course of these two complex first-order reactions can be followed with the stopped-flow method by observing the fluorescence of NADPH: experimentally this is observed as a lag phase followed by a first-order reaction.[42] Detailed analysis of the kinetic data gives a rate constant of approximately $31\ s^{-1}$ for the β-ketoacyl synthase reaction and approximately $17\ s^{-1}$ for the β-ketoacyl reductase reaction (pH 7.0, 25 °C). An independent study of the reduction of acetoacetyl-CoA by NADPH gives a rate constant of about $20\ s^{-1}$ for the β-ketoacyl reductase reaction.[39] The isotope rate effect for this reductase with deuterated NADPH is very small, indicating that hydride transfer is not rate limiting.[44]

No method is available for studying the dehydration of the 3-hydroxybutyryl-enzyme although the dehydratase activity can be assayed with model substrates.[45] The rates of reduction of crotonyl-enzyme and 3-hydroxybutyryl-enzyme by NADPH are identical, suggesting that they are equilibrated rapidly relative to the rate of reduction. As previously indicated, the formation of the 3-hydroxybutyryl- and crotonyl-enzyme from the corresponding CoA derivatives is relatively slow, but their reduction is rapid. The turnover number of the enoyl reductase reaction is approximately $37\ s^{-1}$ at pH 7.0, 25 °C. Therefore, the turnover number for the dehydratase is $>37\ s^{-1}$.

The hydrolysis of the palmitoyl-enzyme has been studied by using palmitoyl-CoA as a substrate (W. Liu and G. G. Hammes, unpublished results). The turnover number is approximately $2\ s^{-1}$. Whether or not this reaction is a good model for the hydrolysis of palmitoyl-enzyme is unknown. As expected, this is a relatively slow reaction: otherwise the growing fatty acid chain might be terminated prematurely.

If the turnover numbers of the individual reactions are assumed to be independent of the fatty acid chain length, the overall turnover number can be estimated (eq 8): $k_{cat} < (1/43 + 7/31 + 7/17 + 7/37 + 1/2)^{-1} = 0.7\ s^{-1}$, with the malonyl transacylase and dehydratase turnover numbers being unknown. The measured steady-state turnover number is $0.8\ s^{-1}$,[34] which is in remarkably good agreement.

An assessment of how the turnover numbers of the individual enzymes vary with the fatty acid chain length can be obtained by examining the distribution of chain lengths covalently bound to the enzyme during catalysis.[32] As previously discussed, if the thioesterase is removed from the enzyme complex, covalently bound intermediates accumulate on the enzyme and the nature of the intermediates can be varied by adjusting the substrate concentrations. The distribution of intermediates can be predicted by simple kinetic models. A detailed analysis suggests that the initial condensation and reduction steps are slower than the analogous reactions with longer chain length intermediates. Although a unique solution cannot be obtained, models in which the rate constants increase by about a factor of 2 as the chain length increases by 2 carbons are consistent with the data. For this model, the calculated value of k_{cat} is $<1.3\ s^{-1}$, which is in reasonable agreement

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with the steady-state value. This increase in rate as the chain length increases insures that the synthesis of palmitic acid is greatly favored over hydrolytic cleavage of covalently bound intermediates.

An interesting feature of the synthesis of palmitic acid is that no clear rate-determining step is present during each cycle of chain growth. (The β-ketoacyl reductase reaction is relatively slow but not rate determining.) In terms of evolutionary development, this provides a process of optimal efficiency since there is no evolutionary advantage to having one step in the cycle much slower than all of the others.

Conclusion

Although the molecular details of the action of fatty acid synthase remain to be determined, the remarkable structure and efficiency of the enzyme complex are apparent. With the availability of the gene for fatty acid synthase, the stage is set for some interesting experiments. Individual structural domains can be expressed, their interactions can be studied, and site-specific mutagenesis can be utilized to determine structure–function relationships.

Registry No. Fatty acid synthase, 9045-77-6.

Interactions between Nonconjugated π-Systems

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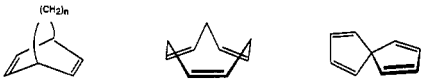
SCF MO theory predicts that two identical π-systems that are coplanar and are connected by a single bond (e.g., the two ethylene fragments that form the π-system of butadiene, Figure 1) interact strongly due to the overlap of the $2p_\pi$ AOs at the termini that are connected to each other. As a consequence, the energies of the resulting bonding and antibonding combinations of the two fragment π- and π*-orbitals will differ: The bonding combinations (π_+, π_+*) are stabilized, and the antibonding combinations (π_-, π_-*) are destabilized. The size of this splitting is a measure of the magnitude of interaction.

Such an interaction is, however, not limited to situations where the π-subunits are directly connected (conjugated). The molecular structure of a number of organic systems can also force π-fragments to overlap through space. This type of interaction has been subdivided into several classes. The most common names that have been introduced are "homoconjugation" and "spiroconjugation". The term homoconjugation[1] is frequently used when both π-fragments (usually olefinic systems) are separated by one or sometimes two CH_2 groups. Prominent examples of this type of through-space interaction are the bicyclo[2.2.*n*] series 1 and 1,4,7-cyclononatriene (trishomobenzene (2)). In spirononatetraene (3) the central atom forces both butadiene fragments perpendicular to each other and thus they can interact through space, providing an example of spiroconjugation.[2]



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In 1968 R. Hoffmann recognized that the interaction between π-units can also be mediated by the σ-framework of the molecule (through-bond interaction[3a,b]). Since then several review articles on the interaction between nonconjugated systems have appeared;[3] therefore we will concentrate on the most recent work. We will also limit our discussion to neutral systems and to spectroscopic results that demonstrate the interaction between their π-moieties. Finally, we will discuss the consequences for the reactivity of systems where such interactions occur.

Experimental Measurements and Interpretations

The methods of choice to study the interactions in nonconjugated neutral π-systems are He(I) photoelectron (PE)[4] and electron transmission (ET)[5] spectros-

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