Chicken liver fatty acid synthetase dimer: evidence of asymmetrical structure from iodoacetamide inhibition studies

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The condensing component of chicken liver fatty acid synthetase is inhibited by a sulfhydryl reagent, iodoacetamide, with a second-order rate constant of 0.23 M⁻¹ sec⁻¹ at pH 7.0 and 0°. Complete inactivation requires the modification of approximately 8 -SH groups per dimer of the enzyme. Quantitation of the extent of inactivation in the presence of 1 mM acetyl CoA (which completely protects the enzyme against inactivation) and in its absence shows that complete inactivation results from the binding of approximately 1.1 mol of carboxamidomethyl residues per dimer. These data are consistent with the proposed functional asymmetry of the enzyme.

Animal liver fatty acid synthetases (FAS) (M₉ >500 000) consist of two polyfunctional polypeptide chains of mol. wt. >250 000 each, with the acyl carrier protein function covalently linked to the polypeptide chains (1). This complex enzyme catalyzes a series of approximately 43 reactions starting with acetyl and malonyl CoA and NADPH before a C₁₆ saturated fatty acid is released. Pigeon, chicken, and rat liver FAS can be dissociated at low ionic strength into two subunits of nearly equal mol. wt. (2), and we initially showed that these subunits catalyze all the component reactions of fatty acid synthesis except the condensation reaction between the enzyme-bound acetyl and malonyl groups (3). This was found to result from the inability of the subunits to transfer the acetyl group to the cysteine-SH site of the condensing component.

A number of articles have appeared in the recent past suggesting that the two polyfunctional polypeptide chains of FAS are structurally identical (4-7); however, the functional characteristics of these subunits have not been demonstrated. In a recent study, Stoops and Wakil (8) have reported that the cysteine-SH site of the condensing component is modified by 1,3-dibromo-2-propanone. Based on the specificity of this reaction and the reported limit stoichiometry of approximately 1.8 mol of the inhibitor bound per dimer, it has been suggested that FAS contains two equally functional condensing sites and that the subunits are functionally identical. Since the condensation reaction requires the participation of both the pantetheine-SH site of the prosthetic group and the cysteine-SH site of the condensing component, reaction at either one or both sites will result in the enzyme inactivation (9). Additionally, since the reagent can react at
either one or both of these sites or at any of the other 40-45 freely accessible -SH groups that react with 5,5'-dithio-bis-(2-nitrobenzoic acid), the reported stoichiometry could have a number of interpretations.

In this communication, we report the stoichiometry of the reaction of chicken liver FAS with another sulfhydryl reagent, iodoacetamide (IAM). Six or 7 of the approximately 45 accessible -SH groups are modified at about the same rate with proportional loss of enzyme activity. Modification in the presence or absence of excess acetyl CoA (which binds at and protects the condensing site) shows that only one of the -SH groups needs to be modified to effect complete inactivation. These data are consistent with our recently published model for the mechanism of action of animal fatty acid synthetases which is based on the functional asymmetry of the polypeptide chains of FAS (10).

Materials and Methods

Enzyme purification

Chicken liver FAS was purified from livers of starved and re-fed birds according to the method of Srinivasan and Kumar (11) and assayed spectrophotometrically as described before (11).

Reaction of the enzyme with IAM

FAS, frozen at -80° in 0.2 M K-phosphate, 3 mM EDTA, 10 mM dithiothreitol, pH 7.0, containing 10% glycerol, was thawed at room temperature and precipitated with one-half its volume of saturated (NH₄)₂SO₄ (33% final saturation). The precipitate was centrifuged, redissolved in the 0.2 M K-phosphate buffer lacking dithiothreitol, and reprecipitated. The process was repeated once more and the pelleted FAS was dissolved in a minimum volume of the above buffer minus dithiothreitol and fractionated on a G-25 Sephadex column. Tubes containing the highest OD₂₈₀ were combined.

For the reaction with IAM, enzyme (0.5 mg/ml) was incubated with 0.5 to 1 mM IAM in 0.2 M K-phosphate, 3 mM EDTA, pH 7.0, at 0° or 25°. Aliquots (20 μl) were withdrawn at various time intervals and diluted into 0.8 ml of the above buffer containing 3 mM dithiothreitol to stop modification and then assayed for overall enzyme activity. The binding of [¹⁴C]IAM in the presence and absence of acetyl CoA (1 mM) was carried out under the same experimental conditions. Aliquots (150 μg) of the enzyme were precipitated at various time intervals with 5% HClO₄; 500 μg of carrier bovine serum albumin was added and the precipitates were filtered on 0.45-μm filter, washed thoroughly with ice-cold 10% TCA, and counted for radioactivity. Corrections were made for non-specific non-covalent binding using two methods: (a) the enzyme was cooled to 0° to which [¹⁴C]IAM and HClO₄ were simultaneously added, or (b) the enzyme was first precipitated with HClO₄ followed by the addition of [¹⁴C]IAM. Calculations of the moles of carboxamidomethyl residues bound per mole of enzyme are based on a molecular weight of 500 000 for the dimer.
Component reactions of fatty acid synthesis

For measurement of component reactions of fatty acid synthesis, enzyme (0.5 mg/ml) was treated with 1 mM IAM in 0.2 M K-phosphate, 3 mM EDTA, pH 7.0, 25°. After 30 min, dithiothreitol was added, to a final concentration of 20 mM. After incubation for an additional 10 min the solution was cooled in ice and dialyzed at 0-4° against 1000 vols. of 0.2 M K-phosphate, pH 7.0, containing 3 mM EDTA and 1 mM dithiothreitol. Assays for partial component reactions were carried out as described before (3).

Results and Discussion

The data on the rate of inactivation of chicken liver FAS at different concentrations of IAM and at different temperatures are given in Fig. 1. Under these conditions, pseudo-first-order plots are obtained. This indicates that inactivation results from the modification of one or more identically reacting groups in the dimer. The

![Graph](image_url)

Fig. 1. Semilogarithmic plot of inactivation of chicken liver fatty acid synthetase by iodoacetamide (IAM). All reactions were carried out at pH 7.0 in 0.2 M K-phosphate, 3 mM EDTA: ○, enzyme 1 mg/ml, IAM 1 mM, 0°; △, enzyme 1 mg/ml, IAM 0.5 mM, 25°; ■, enzyme 0.5 mg/ml, IAM 1 mM, 25°; ×, enzyme 0.5 mg/ml, IAM 1 mM, acetyl CoA 1 mM, 25°.
second-order rate constant calculated at 0° and pH 7.0 is 0.23 M⁻¹ sec⁻¹. This value is approximately 20-fold greater than that for the reaction between free cysteine and IAM and is about one-fifth of the rate constant for the reaction of yeast FAS with IAM at pH 6.5 and 0° (12). Acetyl CoA (1 mM) completely protects the enzyme against inactivation by IAM, which suggests that IAM binds to the same reactive groups which bind acetyl groups during catalysis of fatty acid synthesis. Since the acetyl group binds to the cysteine-SH site of the condensing component and the pantetheine-SH site of the acyl carrier protein component and the modification of either one or both of these sites can result in inactivation, IAM could be reacting at one or both these centers. Though IAM has previously been identified as an inhibitor of the condensing activity of FAS (8,12), the stoichiometry of this inactivation reaction with animal FAS has not been established. As mentioned previously, since the reaction with 1,3-dibromopropanone gives a stoichiometry of approximately 2 as a result of binding exclusively presumably to the cysteine-SH site of the condensing component, this cysteine must, therefore, exhibit enhanced reactivity towards sulphhydryl-modifying agents. Towards this end, we modified chicken liver FAS by [1-¹⁴C]IAM and the results are shown in Fig. 2. The reaction carried out at two different temperatures and at two different enzyme and IAM concentrations reveals almost a linear decrease in enzyme activity with moles of IAM bound. Approximately six moles of IAM are bound for about 85% loss of activity. These
data show that the cysteine-SH site of the condensing component does not possess special reactivity towards this sulfhydryl reagent, as six to eight of these residues react at a similar rate with IAM with incremental loss of activity. However, this does not mean that all six or more of these sites are associated with the catalytic process. To differentiate between the specific and non-specific modification, the binding of $[^{14}C]$IAM was studied in the presence and absence of acetyl CoA (Table 1). Under the conditions of the experiment [0.5 mg/ml enzyme (1 μM) and 1 mM substrate], the substrate-to-enzyme ratio is 1000 and with an estimated $K_a$ of $10^5$ for acetyl transfer (10), complete saturation of the reactive sites is expected. The data of Table 1 show the difference in moles of carboxamidomethyl residues bound at various time intervals in the presence and absence of acetyl CoA. At 20 min where approximately 80% of the activity is lost, the differential moles bound per mole is 0.85. At 30 min when over 95% of the activity is lost, the differential moles bound is approximately one. On a linear scale, this data adjusted for 100% inactivation will give a value of $\alpha$ 1.1 mol of carboxamidomethyl residues bound per

<table>
<thead>
<tr>
<th>Time of incubation</th>
<th>Moles of carboxamidomethyl residues bound per mole of enzyme</th>
<th>Difference (moles per mole)</th>
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<tbody>
<tr>
<td></td>
<td>-AcCoA</td>
<td>+AcCoA</td>
</tr>
<tr>
<td>5</td>
<td>1.39 ± 0.10</td>
<td>1.19 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>3.22 ± 0.20</td>
<td>2.52 ± 0.01</td>
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<td>15</td>
<td>4.34 ± 0.10</td>
<td>3.75 ± 0.02</td>
</tr>
<tr>
<td>20</td>
<td>5.59 ± 0.16</td>
<td>4.74 ± 0.10</td>
</tr>
<tr>
<td>30</td>
<td>7.93 ± 0.02</td>
<td>6.96 ± 0.04</td>
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*Fig. 2. Relationship between the stoichiometry of iodoacetamide (IAM) binding and the loss of activity for fatty acid synthesis. These studies were carried out in 0.2 M K-phosphate, 3 mM EDTA, pH 7.0. The data points correspond to: O, enzyme 1 mg/ml, IAM 1 mM, 0°C; Δ, enzyme 1.0 mg/ml, IAM 0.5 mM, 25°C; and ○, enzyme 0.5 mg/ml, IAM 1 mM, 25°C.*
enzyme dimer. If the two condensing components were functionally identical, stoichiometry of 2 is to be expected. Furthermore, since IAM can also react with the pantetheine-SH and if pantetheine were to react at any significant rate with IAM, one would expect a stoichiometry of greater than 2 if both condensing sites were to be identical. In our data, a stoichiometry of IAM binding of slightly greater than 1 might indicate slight reaction of IAM with the pantetheine-SH site. Table 2 shows the comparison of some of the component reactions of fatty acid synthesis. The loss of condensing activity follows the loss of overall activity for fatty acid synthesis but the activities of the two transacylase reactions are somewhat enhanced. The transacylase activities reflect the rate of transacylase component-catalyzed transfer of the acyl group of acyl CoA to pantetheine-SH in solution which competes with the enzyme-bound pantetheine-SH group. It is possible that the slightly higher rate of the transacylase reactions in the modified enzyme is a reflection of the partial modification of the pantetheine-SH site of the enzyme. Under these conditions, the added pantetheine competes more effectively with the enzyme-bound pantetheine-SH and enhances the competitive transfer of the acetyl group to pantetheine in solution.

The data presented in this report do not support the concept of two independently functional condensing components of chicken liver FAS and are consistent with a model proposed by us for the mechanism of action of animal fatty acid synthetases (10). In our model,

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<tr>
<th>Component reaction</th>
<th>Activity</th>
<th>Percent control activity</th>
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<tbody>
<tr>
<td>Fatty acid synthesis</td>
<td>1528</td>
<td>120</td>
</tr>
<tr>
<td>Acetyl CoA: pantetheine* transacylase</td>
<td>304</td>
<td>382</td>
</tr>
<tr>
<td>Malonyl CoA: pantetheine* transacylase</td>
<td>669</td>
<td>761</td>
</tr>
<tr>
<td>Condensation—CO₂ exchange* reaction</td>
<td>33.4</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*[^14C]acyl CoA, 0.1 mM; pantetheine-SH, 5 mM; and enzyme, 2 μg/ml. Reactions were carried out at 0-4°C in a final vol. of 0.25 ml.
†Malonyl CoA, 0.2 mM; octanoyl CoA, 0.2 mM; CoA, 1 mM; H¹⁴CO₃, 32.5 mM (2000 dpm/mmol); and enzyme, 200 μg/ml. Reactions were carried out at 25°C in a final vol. of 0.25 ml.
the active conformation achieved as a result of covalent transfer of substrates is proposed to be asymmetrical such that one subunit of the dimer acts as a catalyst and the other acts as coordinator during the cycle of reactions of fatty acid synthesis.

In the proposed model, the overall activity for fatty acid synthesis will be inhibited if one mole of the component enzyme is inactivated per dimer. The results of the studies on the inactivation of the condensing component by IAM are consistent with this model.

Acknowledgement

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References