ABSTRACT

The enzymic activity of the GTP-dependent acyl-CoA synthase isolated from rat liver mitochondria is affected by removal and addition of lecithin (3). Purified preparations of this enzyme contain bound lecithin. In this presentation the nature of the lecithin-protein interaction is examined. From binding measurements at different temperatures it is possible to postulate which type of secondary valence bonds is holding the protein and lecithin together.

INTRODUCTION

Two GTP-dependent acyl-CoA synthase preparations have been described, both catalyzing the reaction:

$$R-COOH + \text{CoASH} + \text{GTP} \rightarrow R-COS\text{CoA} + \text{GDP} + \text{Pi}$$

where RCOOH stands for fatty acids; CoASH, reduced coenzyme A; GDP and GTP, guanosine di- and triphosphates, respectively; Pi, inorganic orthophosphate. The first was isolated from acetone powders of beef liver mitochondria (1) and was active with fatty acids up to 12 carbons. The second was prepared from sonically-disrupted rat liver mitochondria and accepted both short and long chain fatty acid substrates (2). Subsequent work demonstrated that lecithin may play an important role in determining the catalytic activity of the second enzyme (3). Purified preparations of this enzyme contained lecithin. Successive extraction of the lyophilized enzyme with 90% acetone diminished enzyme activity. The activity was almost completely restored by adding back the extracted lecithin or purified egg lecithin (3).

The sensitivity to the treatment with organic solvent could explain the differences in substrate specificity between the two enzymes. The beef liver synthase, active on short chain fatty acids only, was purified from an acetone powder of beef liver mitochondria. No organic solvent was used during the purification of rat liver synthase. In other words, endogenous bound lecithin seemed to impose the chain length specificity.

In the present communication the recombination of enzyme protein with lecithin is further quantitated in order to evaluate the nature of this interaction.

MATERIALS AND METHODS

Enzyme activity was routinely assayed with oleate as substrate by measuring CoA sulfhydryl group disappearance and inorganic phosphate formation (2). The incubation mixture was the same as previously described (2). Incubations were carried out under nitrogen for 10 min at 38 C.

The GTP-dependent acyl-CoA synthase was isolated from rat liver mitochondria following procedures previously described (2). The purified enzyme was lyophilized and 16 mg of protein were extracted three times with 10 ml aliquots of 90% acetone at room temperature. Lecithin was purified from egg phospholipids by thin layer chromatography (4). The recombination of the acetone-extracted protein with a relative excess of lecithin was carried out by incubating 0.8 mg of protein with 0.5 mg of lecithin in 0.2 ml of 0.1 M Tris-buffer (pH 7.4) for 30 min. The molar ratio of lecithin to protein, 40:1, was obtained using 20,000 as the molecular weight of the enzyme (2) (Fig. 1).

RESULTS

Only 21% of the initial specific activity remained after extraction of the native enzyme with acetone (Table I). The activity could be restored to a maximum of 80% of the initial full activity by preincubating the acetone-extracted protein for 30 min at 0 C with egg lecithin. By increasing the temperature of incubation, a smaller per cent of the initial full activity was restored (Fig. 1).

On the assumption that the resultant enzymic activity is a function of the percentage of protein saturated with lecithin, thermodynamic calculations on the binding can be made. The Arrhenius law is satisfied (Fig. 1). By resolving the Van't Hoff relation, an
TABLE I

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity</th>
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<tbody>
<tr>
<td>Untreated</td>
<td>5.00</td>
</tr>
<tr>
<td>Acetone extracted-protein</td>
<td>1.05</td>
</tr>
<tr>
<td>Acetone extracted-protein + lecithin</td>
<td>4.00</td>
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</tbody>
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Specific activity of the rat liver mitochondrial GTP-dependent acyl-CoA synthase extracted three times with acetone. Recombination was carried out by preincubation of extracted protein with lecithin for 30 min at 0°C (see Methods and Materials). The molar ratio of lecithin to protein was 40:1. Specific enzyme activities are expressed in terms of μmoles of -SH disappearance per hour per milligram of protein.

**DISCUSSION**

In view of the fact that the extraction of lecithin from the GTP-dependent acyl-CoA synthase can be achieved by using organic solvents under mild conditions, it seems probable that secondary valence bonds are involved between the enzyme protein and the phospholipid molecule. However, the binding of the phospholipid to the protein has the features of an exothermic reaction which gives rise to a lipid-protein complex. The small negative entropy indicates that the complex has a lower number of degrees of freedom, which suggests the possibility of electrostatic bonds between the polar groups of lecithin and the polar groups of protein. The solvation of both of the reacting species (and hydrogen bonding) may regulate water structure and thereby influence the entropy change. In any case the small negative value of the entropy change is more characteristic of the ionization of dipolar ions.

In the GTP-dependent acyl-CoA synthase, lecithin, linked to the protein by electrostatic bonds, may confer chain length specificity through its hydrophobic residues. In other words, long chain fatty acids (in contrast to short chain fatty acids) need the hydrophobic residues of lecithin to facilitate their binding to the enzyme, perhaps through conformational adaptivity of the active sites of the enzyme. Such a conformational adaptivity of binding sites, put forward by Karush in model systems (5), is in agreement with the features of the superstructural components of biological membranes (6).

The binding of phospholipids with mitochondrial enzymes has been discussed extensively by Green and Fleischer (7), who emphasized two types of interaction occurring in mitochondria. The first is mainly hydro-

FIG. 1. Top; Per cent of original enzyme activity (A) vs. temperature of preincubation with lecithin. Lecithin was added to the acetone-extracted protein at a molar ratio of 40:1. The samples were preincubated at the temperatures indicated for 30 min and then assayed as described (2); Bottom; Arrhenius plot, per cent of enzyme activity (A) vs. the reciprocal of the absolute temperature of incubation.

enthalpy change ΔH (negative value) of about -3 kcal/mole and an entropy change ΔS (negative value) of about -12 entropy units have been found. The calculation of the thermodynamic parameters is made by using the following equations:

\[ ΔH = 4.57 T \left( \frac{T_2 \log(A_2/A_1)}{T_2 - T_1} \right) \]
\[ ΔS = 4.57 \left( T_2 \log A_2 - T_1 \log K_1 \right) / (T_2 - T_1) \]

where A is the per cent of initial enzyme activity and T is the absolute temperature.