The Functional Size of Acyl-coenzyme A (CoA):Cholesterol Acyltransferase and Acyl-CoA Hydrolase as Determined by Radiation Inactivation*

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Frozen rat liver microsomes and rough endoplasmic reticulum were irradiated with high energy electrons. The surviving enzymatic activity of acyl-CoA:cholesterol acyltransferase and activity for esterification of 25-hydroxycholesterol decreased as a simple exponential function of radiation exposure, leading to a target size of 170–180 kDa. The loss of acyl-CoA hydrolase activity with a radiation dose was complex and resolved as a 45-kDa enzyme associated with a large inhibitor. It is interpreted that acyl-CoA hydrolase is the acyl-CoA-binding component and the inhibitor is the cholesterol-binding component of acyl-CoA:cholesterol acyltransferase.

Cholesterol is required by all mammalian cells for the proper functioning of cellular membranes. Similarly, almost all eucaryotic organisms require cholesterol or related sterol for cell viability. In addition to the free sterol, cholesterol is found esterified to long chain fatty acids. Cholesteryl ester is thought to be a storage form of cholesterol. In general, the concentration of cholesteryl ester is greatest in those tissues (liver, intestine, ovary) which play a major role in the synthesis and metabolism of cholesterol. Large amounts of cholesteryl esters are known to accumulate in certain tissues as a result of diseases in lipid metabolism such as atherosclerosis.

The intracellular esterification of cholesterol is catalyzed by the enzyme acyl-CoA:cholesterol acyltransferase (ACAT),1 which is a membrane-bound enzyme localized in the endoplasmic reticulum of the cell (1–3). Because of its central role in cellular cholesterol metabolism, there has been great interest in the properties and regulation of ACAT. Although ACAT has been solubilized from microsomes (4, 5), the purification of ACAT has been unsuccessful. Therefore, little is known about the physical properties of ACAT.

Radiation inactivation by high energy electrons is a unique method for determination of the molecular size of a membrane-bound protein without prior purification. It requires only that the activities of interest survive freezing and thawing. We have utilized this method on rat liver microsomes and report here the functional molecular mass of ACAT1 and of the enzyme acyl-CoA hydrolase (ACH). A possible interrelationship between ACAT and ACH is discussed.

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† The abbreviations used are: ACAT, acyl-CoA: cholesterol acyltransferase; ACH, acyl-CoA hydrolase; RER, rough endoplasmic reticulum.
RESULTS AND DISCUSSION

Initial experiments (data not shown) demonstrated that ACAT and ACH activities were not significantly affected by freezing (at −80 °C) and thawing the microsomal or RER preparations; similar stability has been reported previously (15). Glucose-6-phosphate dehydrogenase added to these preparations as an internal standard yielded a target size of 117 kDa (data not shown), in agreement with the value (104 ± 16 kDa) obtained by McIntyre and Churchill (16).

ACAT activity was measured by the incorporation of [14C]oleoyl-CoA into cholesteryl oleate. Radiation inactivation of frozen ACAT was observed both in microsomal preparations and in samples of purified RER. In all cases, the loss of up to 99% of the activity was found to be a simple exponential function of radiation dose (see Fig. 1 and Table I). Target analyses of these data yielded a molecular mass of 137 kDa in both microsomes and purified RER when membrane lipid was the only source of cholesterol. However, endogenous cholesterol is normally insufficient to saturate the enzyme (10). Under nonsaturating conditions, the activity measurement is substrate-limited and can result in artifactual target sizes (8). In the presence of exogenous cholesterol, ACAT activity was increased 3–4-fold (Table I): irradiated samples showed simple exponential losses of activity, yielding target sizes of ~170 kDa, somewhat larger than those without added cholesterol (Table I). In preliminary studies (17), nonlinear radiation inactivation curves of ACAT were reported. No deviations from simple inactivation curves were observed in this study when the enzyme was assayed either in the presence or absence of exogenous cholesterol.

Incubation of irradiated microsomes with labeled 25-hydroxycholesterol permitted the determination of the esterification of this substrate. A simple exponential loss of this 25-hydroxycholesterol esterification activity was observed (Fig. 2). A target size of 187 ± 12 kDa (n = 4) was obtained. Lichtenstein and Brecher (18) have suggested that the esterification of 25-hydroxycholesterol in rat liver microsomes may be catalyzed by ACAT. Using kinetic arguments, Tavani et al. (11) suggested that they may be two separate enzymes. The target size for the enzyme responsible for the esterification of 25-hydroxycholesterol is indistinguishable from the size of ACAT when measured with exogenous cholesterol. This suggests that the esterification of 25-hydroxycholesterol may indeed be catalyzed by ACAT.

The activity of ACH was measured simultaneously with ACAT activity by following the formation of [14C]oleate from [14C]oleoyl-CoA. In both purified RER and microsomal samples, the effect of radiation was complex: at low doses of radiation, there was a small increase in measurable activity, but further radiation exposure reversed this trend, ultimately leading (above 20 megarads) to an apparent exponential decrease in activity. Typical results are shown in Fig. 3 for microsomes assayed without added cholesterol. Assays performed in the presence of exogenous cholesterol showed the same phenomena, but with greater scatter among the data. These results were analyzed as the difference of two exponentials (14). This reflects a model predicated on a smaller enzyme together with a larger structure which blocks or masks its activity. Data from the various preparations and assay conditions indicated an enzyme target size of 46 kDa (Table II). This is in good agreement with the molecular weights of the enzymes.

Data Analysis—Enzyme activities in each sample were normalized for protein. A small fraction of ACAT activity (1%) and of the esterification of 25-hydroxycholesterol (2%) was found to be insensitive to radiation. This quantity, determined at high radiation doses, was subtracted before analysis. The specific activity in irradiated samples was expressed as a fraction of that observed in the nonirradiated control. The logarithm of surviving fraction was plotted as a function of radiation dose (the inactivation curve). For simple exponential inactivation curves, a least-squares analysis constrained to 1.0 at zero dose was performed, and target size calculations were as previously described (8). Complex inactivation curves were analyzed as described elsewhere (14) and reported here as averages ± S.D.

**TABLE I**

<table>
<thead>
<tr>
<th>Assay without exogenous cholesterol</th>
<th>Assay with exogenous cholesterol</th>
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<tr>
<td><strong>kDa</strong></td>
<td><strong>kDa</strong></td>
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<tr>
<td>Microsomes</td>
<td>137 ± 17 (n = 6)</td>
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<td></td>
<td>173 ± 91 (n = 6)</td>
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<tr>
<td>RER</td>
<td>137 ± 14 (n = 5)</td>
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<tr>
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<td>161 ± 13 (n = 7)</td>
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**Fig. 1.** Loss of ACAT activity in irradiated microsomes. Assays were performed without exogenous cholesterol. Typical data are from one experiment of six and were obtained as described under “Materials and Methods.”

**Fig. 2.** Loss of ability to esterify 25-hydroxycholesterol in irradiated microsomes. Data are from one experiment typical of four.
purification of ACAT from rat liver, ACH activity was always observed in the same fractions as ACAT activity. This, along with the apparently large inhibitor of ACAT, suggested that ACH may be involved in the transacylation of acyl-CoA as well as its hydrolysis. If ACAT were composed of different subunits (one involved in the binding of acyl-CoA and the other in the binding of cholesterol), then in the presence of oleoyl-CoA and cholesterol, esterification would occur; in the absence of cholesterol (or if the cholesterol-binding subunit were destroyed), oleoyl-CoA would be hydrolyzed. If the inhibitor protein truly is the cholesterol-binding component of ACAT, then the maximal ACH activity should be equivalent to ACAT activity plus ACH activity at the zero radiation dose. The ratio of the theoretically maximal activity to observed activity is given by: (ACAT activity + ACH activity)/ACH activity. The present data yield a value of 1.4 ± 0.2. In the model, the same value should be obtained by extrapolating the linear portion of the graph (Fig. 3) to zero radiation dose (the maximal ACH activity expressed in the absence of all inhibitor molecules); this yields the intercept error is large, the data are consistent with a two-component model for ACAT/ACH.

Microsomes contain other acyltransferases which are involved in triglyceride and phospholipid synthesis. These enzymes also may be hetero-oligomers in which a common acyl-CoA-binding subunit (ACH) is associated with an additional subunit which binds the second substrate (e.g. glycerophosphate). If substrates for these other acyltransferases were not available, only the hydrolysis of acyl-CoA would occur. This would explain the high ACH activity relative to ACAT activity observed in the microsomes.

The proposed model predicts that maximum acyl-CoA hydrolase activity will be observed in the absence of cholesterol substrate for esterification. Since there is always a small endogenous supply of cholesterol, the acyl-CoA hydrolase activity in these membrane preparations will be somewhat less. With the addition of exogenous cholesterol, there should be a progressive decrease in acyl-CoA hydrolase activity and a progressive increase in ACAT activity. Beyond a cholesterol concentration which saturates ACAT, there will be no further increase in hydrolysis and no further decrease in acyl-CoA hydrolase activity (the remaining hydrolysis activity is presumably due to other transferases and could be reduced still further by the addition of the other transferase substrates). Fig. 4 shows ACH and ACAT activities as a function of increasing exogenous cholesterol supplied in the assay. Over the testable range of cholesterol concentrations, both activities behave as predicted, and the decrease in ACH activity is comparable to the increase in ACAT activity.

These results show that the size of the functional unit for ACAT is much larger than that for ACH, whereas the esterification of 26-hydroxycholesterol requires a structure whose size is indistinguishable from that of ACAT. The complex inactivation curve of ACH is consistent with a two-component model consisting of a 45-kDa subunit which binds acyl-CoA together with a larger structure which inhibits its activity. An acyl-CoA-binding unit and a cholesterol-binding unit are both required for ACAT activity, corresponding to the two structures revealed by radiation inactivation of ACH. This model accurately predicts the behavior of both ACH and ACAT activities when microsomes are assayed in the presence of exogenous cholesterol.

REFERENCES


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