Purification of Protein Fatty Acyltransferase and Determination of Its Distribution and Topology*

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Studies reported from this laboratory have demonstrated that O-glycosidic glycoproteins of salivary, pulmonary, and gastrointestinal origin are acylated by fatty acyltransferase residing in Golgi and microsome-enriched fraction (Slomiany, A., Liu, Y. H., Takagi, A., Laszewicz, W., and Slomiany, B. L. (1984) J. Biol. Chem. 259, 13304–13308). Here we report on the successful purification of this enzyme from rough microsomal membranes of rat gastric mucosa and its identification in a number of diverse tissues and organs, such as heart, liver, pancreas, lung, kidney, salivary glands, and lymphoblasts. The enzymatic activity has been released from the stripped and salt-extracted microsomes with 0.5% Triton X-100 and recovered from 100,000 × g supernatant by affinity chromatography on Cibacron blue F3GA column. The retained fatty acyltransferase protein was selectively displaced from the column with 50 μM palmitoyl-CoA. On nonreducing polyacrylamide gel electrophoresis, the enzymatic activity was associated with a 234-kDa complex, and on sodium dodecyl sulfate polyacrylamide gel electrophoresis, the complex afforded 65- and 67-kDa protein bands. Incubation of microsomes with trypsin prior to enzyme extraction resulted in a 50% inactivation of the fatty acyltransferase and generation of 53- and 55-kDa protein bands, which also had affinity to Cibacron blue F3GA and were displaced from the column together with the active (intact) enzyme. We suggest that the fatty acyltransferase is an integral rough microsomal protein partially exposed to cytosol, which catalyzes the fatty acyl-CoA-protein reaction on the cytosolic side of the rough endoplasmic reticulum and that this enzyme is responsible for processing of the group of protein which are entering rough endoplasmic reticulum-Golgi secretory pathway.

The existence of proteins containing covalently bound fatty acids has been recognized for over twenty years (1, 2), but the early reports fostered the idea that fatty acid acylation of proteins is a rare phenomenon. The more recent findings of palmitic acid esterified to amino acids in Sindbis virus glycoprotein (3) and subsequent investigations of the mammalian, yeast, and bacterial cells (4–7) strongly suggested that fatty acylation of proteins is more common than suspected previously. The different types of fatty acylated proteins and glycoproteins have been linked to many important cellular functions, such as membrane anchorage, growth regulation, morphogenesis, receptor assembly, protease protection, and membrane fusion (8–12). Although many studies suggested the intracellular presence of the enzymes catalyzing the acylation of proteins, their identity has not been verified (13–15). So far, attempts to purify the fatty acyltransferases to homogeneity have been unsuccessful and the investigations were limited to studies of the products assembled in the systems where, instead of pure enzyme, the subcellular fractions containing the enzyme activity were utilized. It is not certain yet whether creation of the fatty acylated proteins reflects action of a single or several specific lipid transferases, but considering the types of linkages and the lipids substrates, it is almost obvious that the N-myristoylation, glycosylphosphatidylinositol linkage, and O-palmitoylation require enzymes with different specificity. In our earlier investigations (16–18), we have shown that O-acylation of the glycosylated and diglycosylated mucin substrates can be accomplished using microsomes from animal and human, normal and pathological tissues as the source of fatty acyltransferase activity. The available information on the synthesis of mucous glycoprotein shows that apomucin core, during its processing in rough endoplasmic reticulum-Golgi path is subjected to con- and posttranslational acylation (19). These studies provided evidence that the microsomal fatty acyltransferase enzyme is involved in the early co-translational and posttranslational acylation of a group of proteins which enter the rough endoplasmic reticulum-Golgi pathway of synthesis, modification, and transport (18–22) and that this enzyme is defective in cystic fibrosis (18, 23, 24). Here, we report the purification to homogeneity of the microsomal fatty acyltransferase, its distribution, and topology. We present evidence that the enzyme activity is associated with 65- and 67-kDa polypeptides and that tryptic digestion of the intact microsomes inactivates the enzyme yielding 53- and 55-kDa proteins. The significance of these findings for delineation of the processes involved in protein translocation across the endoplasmic reticulum during and after translation and the impact on their modification are discussed.

EXPERIMENTAL PROCEDURES

Subcellular Fractionation and Preparation of Microsomal Membranes from Rat Gastric Mucosa—Male Sprague-Dawley rats (200 g body weight) were used to prepare the gastric mucosa microsomes as described earlier (20–22). The gently scraped gastric mucosa was suspended in 2 volumes of TKM buffer (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM MgCl₂) containing 0.25 μM sucrose and homogenized in a motor driven Teflon homogenizer using six strokes at 300 rpm, passed through a nylon screen, and centrifuged at 1500 × g. The postnuclear supernate was centrifuged at 13,000 × g for 10 min to separate the mitochondria and the supernatant layered on a discon-
continuous gradient of 1.50, 1.75, and 2.20 M sucrose in TKM buffer. The gradient was subjected to centrifugation at 150,000 × g for 14 h at 4 °C. Rough microsomes, recovered from 1.75 M sucrose layer, were diluted with equal volume of TKM buffer, layered on 1.3 M sucrose in the same buffer, and centrifuged at 190,000 × g for 2 h at 4 °C. The obtained pellet of rough microsomes was used immediately or stored at −80 °C.

To prepare stripped microsomes, the rough microsomal pellet was resuspended in TK buffer (50 mM Tris-HCl, pH 7.5, 50 mM KCl) to a concentration of 100 A280 units/ml and 0.2 M EDTA, pH 7.0, was added to achieve a final concentration of 3 mM of EDTA/10 A280 units of rough microsomes. Aliquots of 0.5 ml were layered on 12.5 ml gradients of 10–55% sucrose in TKM buffer and centrifuged at 150,000 × g for 14 h. The stripped microsomes were collected at 40–45% sucrose. The recovered fraction was diluted with 2 volumes of TKM buffer, centrifuged at 100,000 × g for 1 h at 4 °C, and the pellet of stripped microsomes recovered. The subcellular fractions of nuclei, mitochondria, Golgi vesicles, and smooth microsomes were diluted with equal volume of TKM buffer, layered on 1.3 M sucrose, and homogenized in a motor-driven Teflon homogenizer (10 strokes at 1000 rpm).

**Solubilization and Purification of Microsomal Fatty Acyltransferase**—The rough microsomes were washed with TKM buffer containing 500 mM KCl (high-salt extraction) to release loosely associated proteins, adjusted to 4 mg/ml protein, 25% glycerol, 0.85% Triton X-100, 2 mM EDTA, 0.17 trypsin inhibitory unit/ml aprotinin in TKM buffer, and incubated with 10% of the weight of a Triton X-100. The suspension was mixed for 30 min at 4 °C and then centrifuged at 100,000 × g for 1 h. The supernatant was diluted with TKM buffer to 0.1% with respect to Triton X-100, 2 mM dithiothreitol, and 0.17 trypsin inhibitory unit/ml aprotinin and applied to the Cibacron blue F3GA column. The running buffer consisted of 20 mM Tris-HCl, pH 7.8, equilibration buffer containing 25% glycerol, 2 mM dithiothreitol, and 0.1% Triton X-100. The column was washed with 5 column volumes of the equilibration buffer and then the bound material was displaced from the affinity medium with 50 mM palmitoyl-CoA in equilibration buffer, followed by 3 column volumes of 5 mM quinidine chloride.

**Treatment of Microsomes with Trypsin**—The rough microsomes, washed with high-salt TKM buffer, were adjusted to 8 mg/ml protein in TKM buffer and incubated at 25 °C for 5 min with 0.002, 0.01, and 0.1 mg/ml trypsin (27). Reaction was stopped by trypsin inhibitor and centrifuged at 100,000 × g for 1 h. The pellet was subjected to solubilization with Triton X-100, centrifuged, and the supernatant was used for the assay of fatty acyltransferase activity and isolation of the fatty acyltransferase by Cibacron blue F3GA chromatography. The details of this procedure are described above.

**Polyacrylamide Gel Electrophoresis**—Nondenaturing PAGE was carried out with 7.5% polyacrylamide gel containing 0.5% Triton X-100. The gels were run in 0.05 M Tris-HCl, pH 7.8, 1% SDS, and 2.5 mM dithiothreitol, 2.5 mM hydroxylamine, pH 8.4, and 0.5% Triton X-100. SDS-PAGE was carried out with 12% gel using Phast system (Pharmacia LKB Biotechnology Inc.) according to the instructions provided in the instrument program.

**Preparation of Acceptor**—Mucus glycoprotein, free of C-terminal nonglycosylated fragment was obtained from the purified mucus glycoprotein by digestion with pepsin. For this, 20 mg of mucus glycoprotein was dissolved in 40 ml of 0.1 M citrate buffer, pH 2.2, and incubated at 37 °C for 72 h under a layer of thymol crystals with pepsin, in an enzyme-to-protein ratio of 1:30 (w/w) (28). The soluble digest was lyophilized, dissolved in 6 M urea, 10 mM sodium phosphate buffer, pH 7.0, and chromatographed on a Bio-Gel A-50 column under the conditions described above. The include containing the mucus glycopeptide was dialyzed, lyophilized, and subjected to equilibrium density gradient centrifugation in CaCl2 (16, 17). Following centrifugation, fractions containing glycopeptide were pooled, dialyzed against distilled water, and lyophilized. The deglycosylated glycoprotein was obtained from the purified mucus glycoprotein by the treatment with trifluoromethanesulfonic acid (29). The deglycosylated glycoprotein was freed of reagents and the released sugars were extracted with ethyl ether followed by dialysis against distilled water. Reduction of disulfide bonds in the mucus glycoprotein was performed with β-mercaptoethanol. For this, mucus glycoprotein was dissolved in 2.5% β-mercaptoethanol, 1% SDS, pH 8.7, incubated at 37 °C for 2 h, and alkylated with 0.4 M iodoacetamide at room temperature for 16 h in the dark. Following dialysis and lyophilization, the glycoprotein was dissolved in 6 M urea, 10 mM sodium phosphate buffer, pH 7.0, and separated from the degraded mucus glycoprotein by chromatography on a Bio-Gel A-50 column under the conditions described above. Fractions containing the included reduced glycoprotein peak were pooled, dialyzed against distilled water, and lyophilized. Removal of the covalently bound fatty acids from the isolated mucus glycoprotein in its intact and modified forms was accomplished with hydroxylamine. The glycoprotein samples were incubated at room temperature for 5 h with 1.0 M hydroxylamine, pH 7.0 (10, 29).

**Product Identification and Isolation of Tryptic Peptides from Mucus Glycoprotein Acceptor Acylated with 14C-Palmitate**—The incubates from 10 assay tubes (2 h at 37 °C with 0.5 μg fatty acyltransferase and 14C palmitoyl-CoA) each containing 50 μg of glycoprotein were combined and precipitated with 10 volumes of 20% trichloroacetic acid in 5% phosphatungstic acid. The precipitate was washed free of acids, delipitated, and subjected to cesium chloride equilibrium density gradient centrifugation (17). Fractions containing 14C-labeled glycoprotein were analyzed on 12% SDS-PAGE using Phast system (Pharmacia LKB Biotechnology Inc.) and electrophoresis conditions provided in the instrument program. Lane 1, flow-through eluted with 20 mM Tris-HCl, pH 7.8, equilibration buffer; lane 2, 0.1% Triton X-100; and 25% (v/v) glycerol; lanes 2–9, successive fractions of protein fatty acyltransferase eluted with 50 μM palmitoyl-CoA in the equilibration buffer; lane 10, Triton X-100 extract of the rat gastric mucosa microsomes; and lane 11, molecular mass markers.

**FIG. 1.** Cibacron blue F3GA column chromatography of the Triton X-100 soluble microsomal proteins of rat gastric mucosa. Aliquots of column fractions were analyzed on 12% SDS-PAGE using Phast system (Pharmacia LKB Biotechnology Inc.) and electrophoresis conditions provided in the instrument program. Lane 1, flow-through eluted with 20 mM Tris-HCl, pH 7.8, equilibration buffer; lane 2, 0.1% Triton X-100; and 25% (v/v) glycerol; lanes 2–9, successive fractions of protein fatty acyltransferase eluted with 50 μM palmitoyl-CoA in the equilibration buffer; lane 10, Triton X-100 extract of the rat gastric mucosa microsomes; and lane 11, molecular mass markers.

1 The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.
mucus glycoprotein were pooled, dialyzed, lyophilized, and hydrolyzed with trypsin (Sepharose-bound) (30). After 5 h, the hydrolyzate was eluted from the column with 10% acetic acid, dried under vacuum, and subjected to SDS-gel electrophoresis and electrophoretic transfer to nitrocellulose. The nitrocellulose membranes were first subjected to radioscanning with a Berthold Linear Analyzer and then B-11 monoclonal antibody was used to detect the C-terminal peptide reactive fragments released from purified mucin (31).

Materials—B-11 monoclonal antibody was raised as described in Ref. 31. The following reagents were obtained commercially: the Sepharose-bound trypsin was from Sigma, Cibacron blue F3GA medium was from Pierce Chemical Co., nitrocellulose filters were from Schleicher and Schuell, [14C]palmitoyl-CoA was from Du Pont-New England Nuclear, and the polyacrylamide gel electrophoresis supplies were from Pharmacia LKB Biotechnology Inc. and Bio-Rad. All other materials were commercial preparations of the highest quality available.

RESULTS

Subcellular Fractionation—Rough microsomes separated from other subcellular organelles on discontinuous sucrose gradient were relatively free of Golgi vesicles. The Golgi marker, sulfotransferase, was enriched in 1.5 M sucrose, and only traces (3–5%) were detectable in rough microsomes recovered from 1.75 M layer. More than 60% of the fatty acyltransferase activity was detected in rough microsomes, and some of the enzyme was also recovered from mitochondria fraction. However, in purified mitochondria (50 μg protein/assay), the fatty acyltransferase activity was not detected.

The fatty acyltransferase activity of the rough microsomes was extractable with Triton X-100, and the solubilized portion contained 75–88% of the total enzyme activity. The solubilized enzyme was labile, but could be stabilized by 25% glycerol, protease inhibitors (32), and storage at −80°C. The solubilized enzyme bound with high affinity to Cibacron blue F3GA medium and was selectively displaced from the column with 50 μM palmitoyl-CoA (Fig. 1). Other acyl-CoAs with different fatty acid chain lengths eluted only partially the enzyme and released from the column other unidentified proteins (Fig. 2). However, the steroyl- and other long chain fatty acyl-CoAs were more specific in eluting the enzyme compared with myristoyl- and acetyl-CoAs. One passage of solubilized rough microsomal fraction through the affinity column afforded a homogenous, 150–160-fold purified enzyme (Table I). The components, which eluted in unbound fraction, and those displaced from the affinity medium with 5 M quanidinium chloride, represented 70 and 29% of Triton X-100 microsomal protein extract, respectively. On the non-denaturing PAGE, the material with fatty acyltransferase activity migrated as a 234-kDa band, whereas on SDS-PAGE it separated into 65- and 67-kDa polypeptides (Fig. 3). The protein electroeluted from the 234-kDa band of non-denaturing PAGE exhibited 50% of the original fatty acyltransferase activity, and the enzymatic activity could not be restored once polypeptides were dissociated.

Characterization of Purified Microsomal Fatty Acrlytransferase—A number of characteristics of the fatty acyltransferase enzyme were similar to those described previously (16, 17). The fatty acyltransferase activity toward the substrates was stimulated by Triton X-100, NaF, and dithiothreitol, whereas MgCl2, MnCl2, and EDTA had inhibitory effect (Table II). At pH 7.4, the fatty acyltransferase catalyzed the transfer of palmitate from palmitoyl-CoA to the deacylated carbohydrate free C-terminal portion of the glycoprotein (Fig. 4). An 8-kDa peptide was observed on SDS-PAGE when the tryptic digests of the intact (lanes 4 and 5) and the [14C]palmitate-labeled glycoprotein (lane 6) were separated, and the peptide from [14C]palmitate-labeled glycoprotein of lane 6 contained radioactive palmitate. The 8-kDa peptides (lanes 4–6) were also recognized by the B-11 monoclonal antibody which reacts with C-terminal nonglycosylated portion of glycoprotein and does not recognize the glycosylated portion of the glycoprotein or that fragment of the peptide core.

Structural and Topological Features of Microsomal Fatty Acrlytransferase and Its Interspecies and Organ Conservation—The fatty acyltransferase activity was not displaced from microsomes by high salt or alkaline pH (25), which qualified the enzyme as the integral membrane protein. The orientation of the enzyme within the microsomal membrane was discerned from the enzyme activity and from the fragmentation

![Fig. 2. Elution of Cibacron blue F3GA bound proteins of Triton X-100 soluble microsomal proteins of rat gastric mucosa](http://www.jbc.org/)

**TABLE I**

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein (mg)</th>
<th>Specific activity (nmol/mg/30 min)</th>
<th>Total activity (nmol/mg/30 min)</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>100,000 X g supernatant of triton X-100 extract</td>
<td>3.0</td>
<td>0.18</td>
<td>0.55</td>
<td>1</td>
</tr>
<tr>
<td>Cibacron F3GA chromatography</td>
<td>0.012</td>
<td>28.02</td>
<td>0.33</td>
<td>156</td>
</tr>
</tbody>
</table>
Purification of Protein Fatty Acyltransferase

123456

ND-PAGE SDS-PAGE

MW x 10^{-3} MW x 10^{-3}

400 140

320 66

230 66

140 14

46 21

14 8

Protein fatty acyltransferase purified by Cibacron blue F3GA column was subjected to nondenaturing PAGE as described under "Experimental Procedures." Following electrophoresis, one lane was stained with silver, and the corresponding regions of the other lane containing the denatured enzyme were cut out and electroeluted for 3 h at 300 V (Amicon micro-electroeluter) in 25 mM Tris, 100 mM glycine containing 0.25% Triton X-100. The eluted enzyme was concentrated to 40 ~1 using Centricon microconcentrators, and 1 ~1 was subjected to SDS-PAGE under reduced condition and the rest for enzyme assay. A and B were silver-stained.

TABLE II

Requirements for rat gastric mucus protein fatty acyltransferase activity

Enzyme activities were measured as described under "Experimental Procedures." The standard assay medium contained in 0.10 ml: 100 μg of deacetylated mucus glycoprotein, 35 μM [1-^{14}C]palmitoyl-CoA, 50 μg of protein, 0.5% Triton X-100, 2 mM dithiothreitol, 25 mM NaF, and 100 mM imidazole HCl buffer, pH 7.4. The standard assay gave the specific activity of 28.

<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>100</td>
</tr>
<tr>
<td>Minus Triton X-100</td>
<td>38</td>
</tr>
<tr>
<td>Minus NaF</td>
<td>41</td>
</tr>
<tr>
<td>Minus dithiothreitol</td>
<td>15</td>
</tr>
<tr>
<td>Plus MgCl₂ (10 mM)</td>
<td>82</td>
</tr>
<tr>
<td>Plus MnCl₂ (10 mM)</td>
<td>69</td>
</tr>
<tr>
<td>Plus EDTA (10 mM)</td>
<td>65</td>
</tr>
</tbody>
</table>

The appearance of new smaller than the fatty acyltransferase polypeptide bands and the reduction of fatty acyltransferase activity suggest that the enzyme is exposed on the cytosolic site and that the trypsin cleaved a 12-kDa fragment from it. This fragment of the enzyme appears to be responsible for fatty acyltransferase activity.

The fatty acyltransferase distribution studies in various tissues and species indicate that the enzyme is present in a variety of tissues, organs, and cells (liver, heart, kidney, pancreas, lungs, salivary glands, and lymphoblasts) and seems to be equally prominent in rat cell lines (Fig. 6, Table III). These findings suggest that the enzyme is not only present in the epithelial cells responsible for the assembly of mucus glycoprotein, but it is also present in the tissues which are active in synthesis and intracellular transport of protein.

FIG. 3. Electrophoresis of purified protein fatty acyltransferase on nondenaturing PAGE and SDS-PAGE. Protein fatty acyltransferase purified by Cibacron blue F3GA column was subjected to nondenaturing PAGE as described under "Experimental Procedures." Following electrophoresis, one lane was stained with silver, and the corresponding regions of the other lane containing the denatured enzyme were cut out and electroeluted for 3 h at 300 V (Amicon micro-electroeluter) in 25 mM Tris, 100 mM glycine containing 0.25% Triton X-100. The eluted enzyme was concentrated to 40 ~1 using Centricon microconcentrators, and 1 ~1 was subjected to SDS-PAGE under reduced condition and the rest for enzyme assay. A and B were silver-stained.

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FIG. 4. Identification of the acylated 8-kDa C-terminal peptide of mucus glycoprotein using B-11 monoclonal antibody.

Intact mucus glycoprotein and [14C]palmitoyl glycoprotein were prepared as described under "Experimental Procedures" and subjected to tryptic digestion. Tryptic digests were dried and subjected to 8~25% SDS-PAGE in duplicate. Protein bands from one SDS-PAGE were transferred to nitrocellulose membrane and the other SDS-PAGE was silver-stained. The nitrocellulose membrane was first subjected to radioscanning and then to Western blot analysis using B-11 monoclonal antibody to detect the C-terminal peptide released from mucus (31). Top, silver-stained gel. Lanes 1 and 2, low molecular mass markers; lane 3, mucus glycoprotein deproteinized of the C-terminal peptide following pepsin digestion; lanes 4 and 5, tryptic digest of mucus glycoprotein (27) and lane 6, tryptic digest of [14C]palmitate glycoprotein. The 8-kDa band of lanes 4~6 reacted with B-11 monoclonal antibody. Bottom, linear scan of nitrocellulose membrane using Berthold Linear Analyzer. The membrane was counted for a period of 10 min/lane, the scale indicates counts/min and the radioactive peak of 7.5~8.5 cm corresponds to the 8-kDa tryptic peptide identified in lane 6.

of the enzyme protein when the intact microsomes were subjected to proteolytic digestion. The effect of trypsin on the activity of fatty acyltransferase recovered from such microsomes is shown in Fig. 5A, and the fragmentation of the enzyme protein recovered from the membranes is shown in Fig. 5B. The effect of the microsome digestion with trypsin on the fatty acyltransferase activity revealed that 5-min incubation at 25 °C with 0.1 mg/ml trypsin caused 50~60% reduction in transferase activity and partial destruction of the protein present on the cytosolic site of the microsome. On SDS-PAGE, the trypsin-treated microsomes afforded, in addition to the 65~67-kDa bands, two polypeptides with estimated masses of 53~55 kDa. The appearance of new smaller than the fatty acyltransferase polypeptide bands and the reduction of fatty acyltransferase activity suggest that the enzyme is exposed on the cytosolic site and that the trypsin cleaved a 12-kDa fragment from it. This fragment of the enzyme appears to be responsible for fatty acyltransferase activity.

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DISCUSSION

The data presented in this study detail the purification, topology, and occurrence of the rough endoplasmic reticulum fatty acyltransferase enzyme. This enzyme was partially purified from normal and cystic fibrosis tissues earlier (16, 17, 23) and found to be more active in cystic fibrosis than in healthy individuals (18, 23). On the other hand, much lower...
Purification of Protein Fatty Acyltransferase

1.25

12345 67

0.002 0.01

TRYPSIN CONCENTRATION (mg/ml)

FIG. 5. A, reduction in protein fatty acyltransferase activity by treatment of rat gastric mucosa microsomes with trypsin. Microsomes (1 mg/ml) were preincubated for up to 5 min at 25 °C with varying concentrations of trypsin before extraction of the protein fatty acyltransferase with Triton X-100 and assaying enzyme activity. The inset shows 10- and 20-min time course of protein fatty acyltransferase activity at various trypsin concentrations. The arrow indicates the sample which is depicted on SDS-PAGE gel in Fig. 4B. B, SDS-PAGE of the protein fatty acyltransferase purified from microsomes treated with trypsin. The Triton X-100 extract from microsomes treated with trypsin (0.1 mg/ml) was fractionated on Cibacron blue F3GA column. The material eluting with 50 μM palmitoyl-CoA in equilibration buffer is shown in lanes 2 and 3. The dot indicates the intact enzyme, and the arrow shows the trypsin-resistant portion of the fatty acyltransferase in the intact microsomes.

activity of this fatty acyltransferase was observed in gastrointestinal pathologies (33) and its temporary induction following acute consumption of ethanol (17, 34). These lines of evidence led to the conclusion that the enzyme has a profoundly important function in the modification, processing, and conditioning of O-glycosidic glycoproteins to the survival in the hostile gastrointestinal environment. The fatty acyl residues on the glycoprotein have been regarded as primary factors in supporting the interaction of the glycoproteins with other secretory constituents to form a surface-protective, protease-resistant, and permselective epithelial barrier.

In the light of our clinically relevant findings, and the fact that the enzyme, which is involved in acylation of many other proteins, has never been purified and characterized, we mobilized our efforts to isolate, purify, and characterize this microsomal protein. Rough microsomes were stripped with high salt concentration and EDTA, and from washed microsomal membranes the fatty acyltransferase was released with 0.5% Triton X-100. The Cibacron blue F3GA affinity chromatography was found to give the best separation of the enzymatic activity from other detergent-solubilized components of microsomal membrane. The fraction eluting with 50 μM palmitoyl-CoA contained the fatty acyltransferase activity. On SDS-PAGE, the active material separated into two closely running polypeptide bands in molecular mass range of 65 and 67 kDa. In all steps that followed initial purification, the two polypeptides copurified with fatty acyltransferase activity. Even when microsomes were preincubated with trypsin, the material eluted with palmitoyl-CoA consisted of two doublets; one corresponding to 65-67-kDa enzyme protein and the other migrating in the region of 53-55 kDa. Appearance of shorter polypeptides, with the same affinity to Cibacron blue F3GA as the undegraded enzyme, suggest that both polypeptides were cleaved at about the same place and that the cytosol-exposed fragments of each peptide were similar or perhaps identical to each other. Thus, the 2-kDa difference in molecular mass of the enzyme forms should be within the protected region or the fragment exposed on luminal site of the microsomes. This raises the possibility that one polypeptide might be glycosylated or that there are two differently glycosylated forms of the same protein. The fact that the...
electroeluted 234-kDa complex retains enzymatic activity, whereas the individual subunits are inactive, suggest that both peptides are required for the catalytic activity. Separation, peptide mapping, and amino acid sequencing of these polypeptides should clarify this ambiguity and determine whether the isolated proteins represent the same or closely related enzyme proteins of which one may be responsible for N-terminal cotranslational (19–22) and the other for C-terminal posttranslational acylation (10, 28) of proteins. The proteolytic degradation of the enzyme during preparation is unlikely, since a mixture of protease inhibitors was present during the membrane isolation and solubilization steps and since proteolytic cleavage of the microsomal membranes afforded forms which were 10–12 kDa smaller than the bands observed in untreated and protease-protected microsomes. Also, the endogenous degradation produced 12 kDa smaller peptides. This was observed in the case of the enzyme preparation from pancreas (Fig. 6). In that instance, it appeared as if insufficient amount of protease inhibitors were added during enzyme purification.

Another feature furnished by this investigation is the distribution of the fatty acyltransferase in subcellular fractions and in the functionally different tissues, organs, and cells. Previously, we have shown that the fatty acyltransferase activity was present in functionally different areas of the stomach, intestines, and also displayed by bronchial tissues and salivary glands (22, 23, 33, 35, 36). All these tissues have in common the ability to synthesize O-glycosidic mucin-type glycoproteins. In the present studies, we have shown that the 65- and 67-kDa protein, which display fatty acyltransferase activity, is also present in various tissues, organs, and cells which are not involved in the synthesis of mucus glycoproteins. In addition, we have found that these proteins are only present in microsomal membranes. The purified plasma membranes, mitochondria, Golgi, smooth microsomes, and high-speed cytosol supernatant do not contain fatty acyltransferase activity nor do they contain the 65- and 67-kDa protein bands with affinity to the Cibacron blue column. These results indicate that the fatty acyltransferase enzyme is unique for rough microsomal membrane and represents an integral part of this organelle structure and appears to be distributed in cells involved in the synthesis and processing of polypeptides tracing the rough endoplasmic reticulum–Golgi system.

This study, besides demonstrating the common occurrence of the acyltransferase enzyme and the restrictions with regard to topology, also provides information on its substrate specificity which was tested with decapylated and decysylated and deglycosylated mucus glycoprotein substrates. The important feature of the substrate for this enzyme appears to be a particular amino acid sequence surrounding the acyl-accepting residue and not the entire molecule or glycosylation of the molecule. We still do not know what this sequence might be, but the C-terminal peptides of mucus glycoprotein contain several free serine residues, and yet only one or two in normal glycoprotein are acylated with fatty acids. This and the fact that the truncated glycoproteins (deficient of C-terminal 6-kDa peptide) fail to serve as acceptors of palmitic acid from palmitoyl-CoA suggest that the fatty acyltransferase requires a specific sequence on the C-terminal of the protein acceptor. This enzyme is also responsible for acylation of membrane transport protein (64-kDa chloride channel protein).2 Considering the recent reports that fatty acyl-CoA stimulates intracellular transport (12) and acts as cofactor for Golgi-associated protein termed NSF (37–39) and the fact that a large number of proteins undergo fatty acylation (40), it is tempting to speculate that the fatty acyltransferase characterized here is responsible for the final processing of the proteins in endoplasmic reticulum and that this enzyme plays an important role in facilitating the transport of proteins from endoplasmic reticulum to Golgi.

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