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Degradation of Stearoyl-Coenzyme A Desaturase: Endoproteolytic Cleavage by an Integral Membrane Protease

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Stearoyl-coenzyme A desaturase (SCD) is a key regulator of membrane fluidity, turns over rapidly, and represents a prototype for selective degradation of resident proteins of the endoplasmic reticulum. Using detergent-solubilized, desaturase-induced rat liver microsomes we have characterized a protease that degrades SCD. Degradation of SCD in vitro is highly selective, has a half-life of 3–4 h, and generates a 20-kDa C-terminal fragment of SCD. The N terminus of the 20-kDa fragment was identified as Phe^{177}. The cleavage site occurs in a conserved 12-residue hydrophobic segment of SCD flanked by clusters of basic residues. The SCD protease remains associated with microsomal membranes after peripheral and lumenal proteins have been selectively removed. SCD protease is present in normal rat liver microsomes and cleaves purified SCD. We conclude that rapid turnover of SCD involves a constitutive microsomal protease with properties of an integral membrane protein.

INTRODUCTION

The lipid composition of cellular membranes is regulated to maintain membrane fluidity. A key enzyme in this process is stearoyl coenzyme A (CoA) desaturase (SCD), a Δ^9 desaturase and the rate-limiting enzyme in the synthesis of monounsaturated fatty acids. Poikilothermic animals maintain membrane fluidity in response to cold by up-regulating SCD activity and increasing the unsaturation of membrane phospholipids (Tiku *et al*., 1996). SCD activity is induced in mammals when dietary fat is restricted (Oshino and Sato, 1972) and also by insulin, carbohydrates, and peroxisome proliferators (reviewed in Ntambi, 1995). Animals preferentially use exogenous unsaturated fatty acids and express low levels of SCD when sufficient unsaturated fat is available from dietary sources. The mechanism of SCD induction has been studied extensively and involves transcriptional as well as posttranscriptional controls (reviewed in Ntambi, 1995). Recently, a family of membrane-bound, proteolytically activated transcription factors was identified that regulates multiple genes involved in cholesterol and fatty acid metabolism, including SCD (Brown and Goldstein, 1997). By contrast, little is known about SCD degradation. It was shown some 25 years ago that SCD is degraded rapidly in vivo (Oshino and Sato, 1972). Although most resident proteins of the endoplasmic reticulum have half-lives of 2–6 d (Omura *et al*., 1967; Arias *et al*., 1969), the half-life of SCD is 3–4 h (Oshino and Sato, 1972). SCD is selectively degraded in vitro when microsomes from rats induced for SCD are incubated at 37°C (Ozols, 1997). Degradation of SCD in vitro is not inhibited by a wide variety of protease inhibitors (Ozols, 1997), suggesting that SCD may be degraded by a unique protease.

We have extended our previous observations by developing procedures to solubilize SCD and the microsomal components that degrade it. Using purified SCD and detergent-solubilized microsomes we have reconstituted the microsomal system that degrades SCD in vitro. We show that the microsomal proteases that degrade SCD are highly selective and resist solubilization by procedures that solubilize peripheral and

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lumenal endoplasmic reticulum (ER) proteins. During degradation, a 20-kDa C-terminal fragment of SCD is generated. The cleavage site was identified as the Phe–Phe bond at residues 176–177.

MATERIALS AND METHODS

Preparation of Microsomes

Desaturase-induced microsomes (DSIMs) were prepared as described (Strittmatter and Enoch, 1978) with modifications. Ten male Sprague Dawley rats (Charles River, Wilmington, MA) were starved for 48 h, fed normal laboratory chow for 48 h, starved for a second 48-h period, and refed with Nutritional Biochemical (Cleveland, OH) Fat Free test diet for 20 h on a schedule that permits the animals to be killed at the beginning of a day. All procedures were carried out at 0–5°C. Livers were quickly excised and placed in 800 ml 0.25 M sucrose, 10 mM Tris-acetate (pH 8.1), 1 mM EDTA, and 1 mM dithiothreitol (DTT). The livers were blotted, weighed, minced, and homogenized with 5 ml of sucrose buffer per gram of liver in a Potter-Elvehjem (Kontes, NJ) tissue grinder. The homogenate was centrifuged at 960 × *g* for 10 min. The first supernatant was centrifuged at 18,000 × *g* for 15 min. The second supernatant was carefully separated from the mitochondrial pellet and centrifuged at 92,000 × *g* in a Beckman type 45 rotor for 90 min. The microsomal pellets were suspended in 20% glycerol, 10 mM Tris-acetate (pH 8.1), 1 mM EDTA, and 1 mM DTT using a volume of buffer equal to the original weight of liver. "Control" microsomes were prepared in the same manner from rats that were refed normal laboratory chow for 20 h after a 48-h period of starvation. Microsomes were used immediately or after brief periods of storage at −80°C

Solubilization of SCD and SCD Protease (SCDP)

10% (wt/vol) stock solutions of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (CHAPS; Pierce Chemical, Rockford, IL), Triton X-100 (TX100; Bio-Rad, Hercules, CA), digitonin (Wako, Richmond, VA), and deoxycholate (DOC; Sigma, St. Louis, MO) were prepared in 10 mM Tris-acetate (pH 8.1). A mixed detergent stock solution (10% DOC/TX100) was prepared by combining equal volumes of the DOC and TX100 stock solutions. One milliliter of microsomes was diluted with 0.25 ml of 10% detergent. After 30 min at 4°C, the insoluble material was pelleted by centrifugation at 105,000 × *g* for 60 min in a Beckman (Palo Alto, CA) TLA 100.2 rotor.

Time Course of SCD Degradation

Ten milliliters of DSIMs were diluted with 2.5 ml of 10% DOC/TX100. After 30 min at 4°C, insoluble proteins were pelleted by centrifugation at 105,000 × *g* in a Beckman type 60 rotor for 60 min. One hundred-microliter aliquots of the supernatant were incubated at 37°C.

Purification of SCD

SCD was purified from DSIMs by differential detergent extraction and ion exchange chromatrography as described (Strittmatter *et al.*, 1974; Strittmatter and Enoch, 1978).

Preparation of Salt-washed DSIMs

Twenty-five milliliters of DSIMs were diluted with 25 ml of 1 M NaCl, 10 mM Tris-acetate (pH 8.1) 30 mM EDTA, and 1 mM DTT. After 30 min at 4°C, the membranes were concentrated by centrifugation in a Beckman type 45 rotor at 125,000 × *g* for 65 min. The microsomes (volume, 2.5 ml) were diluted with 100 ml of 10% glycerol, 5 mM Tris-acetate (pH 8.1), 0.5 mM EDTA, and 0.5 mM DTT. After 15 min at 4°C, the membranes were pelleted by centrifugation at 125,000 × *g* in a Beckman type 45 rotor for 35 min. The salt-washed microsomes were suspended in 25 ml of 20% glycerol, 10 mM Tris-acetate (pH 8.1), 1 mM EDTA, and 1 mM DTT.

Preparation of DSIMs Depleted of Lumenal Proteins

Two-hundred microliters of DSIMs were labeled with 20 μl [^{3}H]diisopropylfluorophosphate (DFP; New England Nuclear, Boston, MA; 8.4 Ci/mmol, 0.12 nmol/μl in propylene glycol) for 1 h at 4°C. Fifty microliters of 10% CHAPS were added, and after 30 min at 4°C, the insoluble fraction was collected by centrifugation at 105,000 × *g* for 1 h in a Beckman TLA 100.2 rotor. The CHAPS-insoluble material was suspended in 200 μl of 20% glycerol, 10 mM Tris-acetate (pH 8.1), 1 mM EDTA, and 1 mM DTT and solubilized by the addition of 50 μl of 10% DOC/TX100.

Preparation of Peripheral and Lumenal Proteins

The first supernatant from the procedure to prepare salt-washed microsomes was dialyzed against 10% glycerol, 10 mM Tris-acetate (pH 8.1), 1 mM EDTA, and 1 mM DTT. The dialyzed preparation was centrifuged at 125,000 × *g* for 60 min in a Beckman type 45 rotor. DOC/TX100 (10%) was added to the supernatant to bring the detergent concentration to 2%. Lumenal proteins were selectively extracted with 0.1% TX100. A stock solution of 1% TX100 was prepared in 10 mM Tris-acetate (pH 8.1); 0.1 ml 1% TX100 was added to 0.9 ml DSIMs while vortexing. After 30 min at 4°C, the permeabilized membranes were sedimented by centrifugation at 105,000 × *g* for 60 min. The detergent concentration of the supernatant was adjusted to 2% DOC/TX100.

Assay of SCD Degradation

Degradation of SCD was assayed by following the disappearance of the 37-kDa SCD protein bond by SDS-PAGE. Gels were stained with Coomassie blue. Except where indicated the resolving gels were 10% acrylamide. For each assay, equal aliquots (25–100 μl) of the sample were incubated at 37°C and frozen at −20°C. Degradation was terminated by the addition of SDS-PAGE sample buffer.

Isolation of the 20-kDa Fragment of SCD

Salt-washed DSIMs were solubilized by adding one-fourth volume of 10% TX100. The sample was incubated on ice for 15 min and centrifuged at 114,000 × *g* for 30 min. Fifty milliliters of TX100 extract were incubated at 6–8°C overnight. The extract was passed over a DEAE-cellulose column (volume, 75 ml) equilibrated in 10 mM Tris acetate (pH 8.1), 20% glycerol, 2% TX100, 1 mM EDTA, and 0.1 mM DTT. The column was washed with 100 ml equilibration buffer, and 2-ml fractions were collected. The 20-kDa fragment in effluent fractions was identified by immunoblotting with polyclonal antibody against the C terminus of SCD.

Micro sequence analysis of the 20-kDa band, eluted from a preparative electrophoresis gel with 40 mM ammonium bicarbonate and 0.02% SDS buffer using the Elutrap chamber (Schleicher & Schuell, Keene, NH), according to their instructions, was carried out on an Applied Biosystems (Foster City, CA) 470A gas phase sequenator equipped with a 120A PTH analyzer, according to the manufacturer's instructions.

Antibodies and Immunoblots

Monoclonal antibody to Golgi 58 kDa was purchased from Sigma (G-2404). Polyclonal antiserum against SCD was prepared as described (Ozols, 1997). Polyclonal antiserum against the C terminus of SCD was prepared using a 20-residue synthetic peptide corresponding to residues 338–358 of SCD as antigen. The sequence of the synthetic peptide was based on the cDNA sequence of rat SCD (Thiede *et al.*, 1986). Protein samples were resolved by SDS-PAGE and electrophoretically transferred to sheets of nitrocellulose. The

blots were blocked with 3% albumin. The blots were incubated with antibody overnight at 4°C. Bound antibody was detected with an anti-IgG-alkaline phosphatase conjugate (Sigma A-3687) and a phosphatase detection kit (Kirkegaard and Perry Laboratory, Gaithersburg, MD).

RESULTS

Solubilization and Reconstitution of SCD Degradation

The current studies were designed to establish the solubility characteristics of the microsomal proteolytic system that degrades SCD, to reconstitute SCD degradation using purified SCD and solubilized microsomal protease, and to identify proteolytic fragments of SCD that result from degradation. Initially we investigated the effect of various detergents and salts on SCD degradation. None of the detergents investigated inhibited SCD degradation; however, 500 mM NaCl appeared to inhibit degradation. We therefore performed our solubilization studies in low-ionic-strength buffer. Figure 1 shows an experiment in which DSIMs were solubilized with various detergents. The insoluble fraction was removed by ultracentrifugation, and the detergent supernatants were incubated at 37°C. The SDS-PAGE protein profiles of the detergent extracts before and after incubation are compared in adjacent lanes. Only the detergent combination of DOC/TX100 effectively solubilized SCD (Figure 1, lane 8). After incubation, SCD was depleted from the DOC/TX100 detergent extract indicating that the SCDP was also solubilized (Figure 1, compare lanes 8 and 9). Unexpectedly, a 96-kDa protein was also degraded upon incubation at 37° (Figure 1). This protein is not degraded when DSIMs are incubated in the absence of detergent (Ozols, 1997).

Figure 2 shows a time course of SCD degradation in the DOC/TX100-soluble fraction of DSIMs. SCD is noticeably depleted after 4 h and nearly gone after 12 h. The 96-kDa protein appears completely degraded in 4 h. Quantitation of the data in Figure 2 by image analysis indicated that the half-life of SCD is 3–4 h and the half-life of the 96-kDa protein is 1–2 h. The selectivity of SCD degradation after solubilization with DOC/TX100 suggests that this in vitro system represents a physiologically relevant process. Furthermore, the half-life of SCD in vitro is identical to the half-life reported for in vivo degradation (Oshino and Sato, 1972).

The experiments of Figure 3 were conducted to determine whether proteolytic intermediates of SCD could be detected during degradation and whether degradation could be reconstituted from solubilized components. DSIMs and control rat liver microsomes (CRLMs) were solubilized with DOC/TX100, and the insoluble fraction was removed by ultracentrifugation. Where indicated, the soluble fraction of CRLM

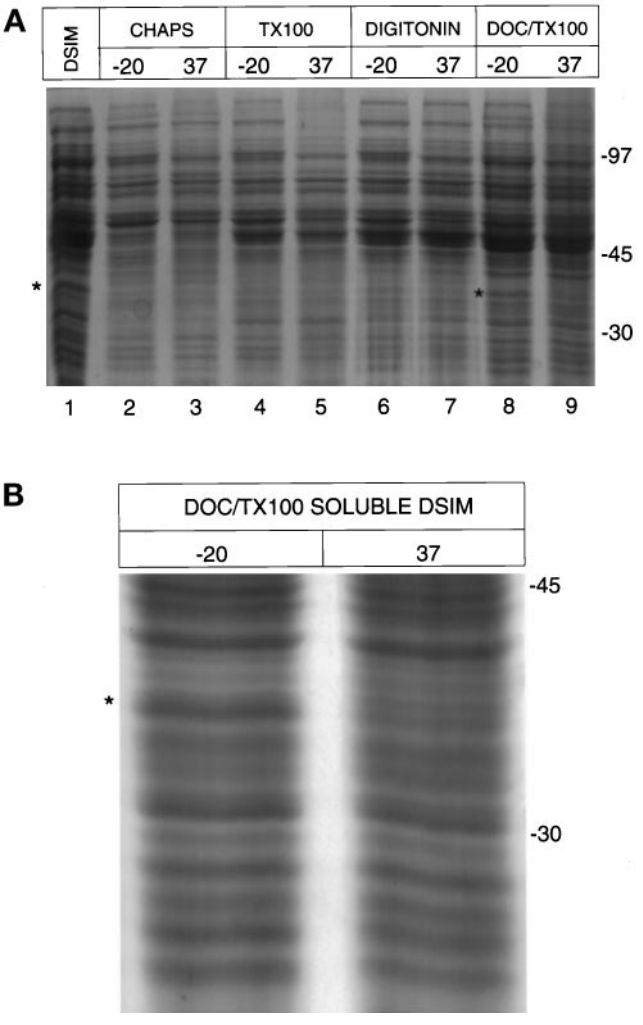

Figure 1. Solubilization of SCD and SCDP. DSIMs were treated with detergent (final concentration, 2%, as described in MATERIALS AND METHODS). Insoluble proteins were removed by ultracentrifugation. The detergent extracts were incubated overnight at 37°C. Controls were frozen at −20°C overnight. The solubilized proteins were separated by SDS-PAGE and visualized by staining with Coomassie blue. (A) The samples analyzed were unfractionated DSIMs (lane 1), CHAPS-soluble DSIMs at −20°C (lane 2) and 37°C (lane 3), TX100-soluble DSIMs at −20°C (lane 4) and 37°C (lane 5), digitonin-soluble DSIMs at −20°C (lane 6) and 37°C (lane 7), and deoxycholate/TX100 (1:1)-soluble DSIMs at −20°C (lane 8) and 37°C (lane 9). The 37-kDa SCD protein band is marked by an asterisk in lanes 1 and 8. The positions of molecular mass markers (kilodaltons) are shown on the right. (B) Enlarged image of the bottom half of lanes 8 and 9. The 37-kDa SCD band is marked by an asterisk on the left. The positions of molecular mass markers (kilodaltons) are indicated on the right.

was supplemented with purified SCD. After incubation the preparations were subjected to electrophoresis and blotted with antisera to SCD. This antisera detected three protein bands in solubilized DSIMs before incubation (Figure 3, lane 1). The largest and most abundant of these represents intact SCD; two minor

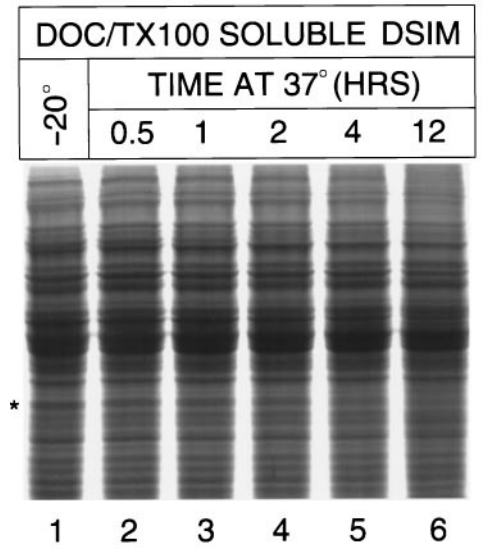

Figure 2. Time course of SCD degradation. DSIMs were solubilized with DOC/TX100. Insoluble proteins were removed by ultracentrifugation. The soluble protein fraction was incubated at 37°C for the indicated times, subjected to SDS-PAGE, and stained with Coomassie blue. Time points analyzed were 0 h (lane 1), 0.5 h (lane 2), 1 h (lane 3), 2 h (lane 4), 4 h (lane 5), and 12 h (lane 6). The SCD protein band is marked with an asterisk to the left of lane 1.

bands with apparent molecular masses of 35 and 32 kDa result from loss of material at the N terminus of SCD. Several residues from the N terminus of the SCD molecule can be deleted without affecting the catalytic activity of the enzyme (Ozols, 1997). After incubation, three new bands were detected with apparent molecular masses of 24, 23, and 20 kDa (Figure 3, lane 2). CRLMs contained low levels of SCD (Figure 3, lane 3). No proteolytic intermediates were detected in solubilized CRLMs before or after incubation (Figure 3, lane 4). However, when the CRLM preparation was supplemented with purified SCD, the 20-kDa proteolytic fragment was detected after incubation (Figure 3, lane 6). To further characterize the proteolytic fragments of SCD, antisera were prepared against a C-terminal peptide of SCD. The C-peptide antisera detected the 20-kDa fragment of SCD generated during degradation (Figure 3B, lane 8). These results suggest that a constitutive microsomal protease cleaves SCD and generates a 20-kDa C-terminal fragment. To detect the 20-kDa intermediate by immunoblot, we loaded a relatively large amount of sample on the gel. Under such conditions, there is no visible difference in the amount of 37-kDa band detected by immunoblot after incubation, even though there is a marked decrease in the amount of 37-kDa band detected by Coomassie blue staining. If a smaller amount of sample is loaded, a decrease in the 37-kDa band can be demonstrated by immunoblot, but then the 20-kDa fragment is not detected. The ranges in which the two methods are quantitative do not even overlap. In fact, the maximum immunoblot signal is produced by a quantity of SCD that is below the limit of detection by Coomassie blue staining.

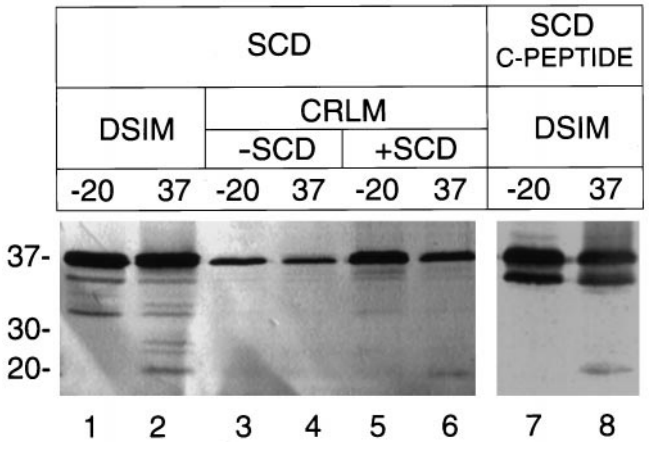

Figure 3. Immunoblot analysis of SCD degradation. Nitrocellulose blots were probed with rabbit antiserum to SCD (lanes 1–6) and rabbit antiserum to a C-terminal peptide of SCD (lanes 7–8). The samples analyzed were the DOC/TX100-soluble fraction of DSIMs before (lanes 1 and 7) and after (lanes 2 and 8) incubation at 37°C and the DOC/TX100-soluble fraction of CRLMs unsupplemented (lanes 3 and 4) and supplemented with 2 μg purified SCD (lanes 5 and 6) before (lanes 3 and 5) and after (lanes 4 and 6) incubation at 37°C.

SCDP Is an Integral Membrane Protein

Microsomal proteins can be separated into three groups based on their solubility characteristics. Peripheral proteins bound to the cytosolic membrane surface by ionic interactions are selectively solubilized by aqueous buffers that contain sufficient salts. For example, the signal recognition particle is efficiently extracted from rough microsomes with solutions of high ionic strength (Warren and Dobberstein, 1978). Lumenal proteins are selectively solubilized by freezing and thawing microsomes or by detergents used below their critical micelle concentration (Kreibich and Sabatini, 1974). Integral membrane proteins typically require detergent at concentrations above the critical micelle concentration for solubilization (Hjelmeland, 1990). The experiment of Figure 4 was conducted to determine whether SCDP is a peripheral protein. DSIMs were washed with 500 mM NaCl/15 mM EDTA to strip off peripheral proteins. The salt-washed microsomes were then washed with buffer to reduce the NaCl concentration and incubated overnight at 37°C. Incubation of salt-washed DSIMs led to the complete disappearance of the 37-kDa band from the SDS-PAGE gel profile (Figure 4A). Immunoblot analysis of salt-washed DSIMs with SCD antibody

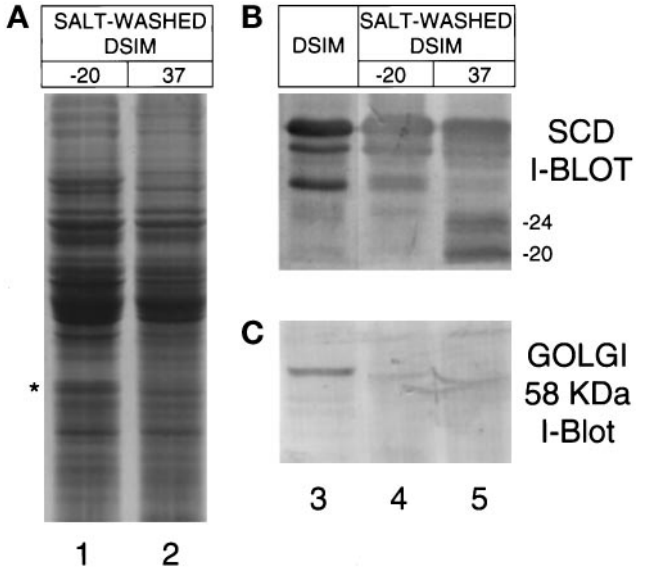

Figure 4. Degradation of SCD in salt-washed DSIMs. Peripheral membrane proteins were extracted from DSIMs with NaCl/EDTA as described in MATERIALS AND METHODS. The salt-washed microsomes were solubilized in DOC/TX100 and incubated overnight at 37°C. (A) Coomassie blue-stained gel of salt-washed DSIMs before (lane 1) and after (lane 2) incubation. An asterisk marks the 37-kDa SCD band in lane 1. (B and C) Immunoblot analysis of DSIMs (lane 3) and salt-washed DSIMs (lanes 4 and 5) before (lane 4) and after (lane 5) incubation. Nitrocellulose blots were probed with rabbit antiserum to SCD (B) and monoclonal antibody to Golgi 58-kDa protein (C).

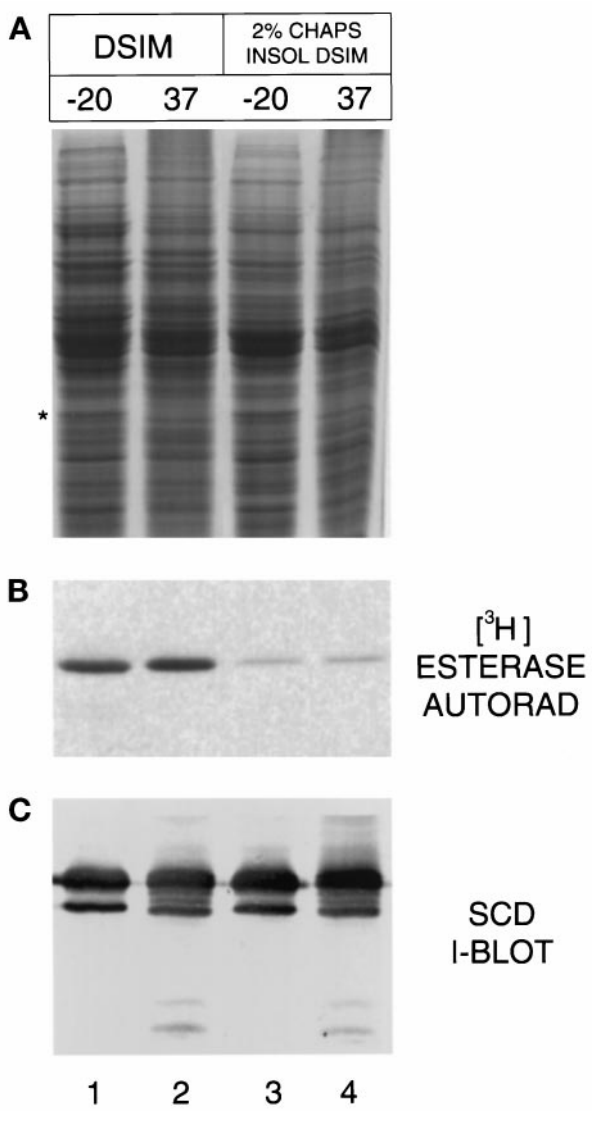

Figure 5. Degradation of SCD in DSIMs depleted of lumenal proteins. DSIMs were incubated with [^{3}H]DFP to label the lumenal esterases. The labeled DSIMs were solubilized with 2% CHAPS. The insoluble protein fraction was collected by ultracentrifugation, suspended in buffer containing 2% DOC/TX100, and incubated overnight at 37°C. The samples were analyzed by Coomassie blue-stained SDS-PAGE (A), autoradiography (B), and immunoblot (C) using rabbit antiserum to SCD. The samples analyzed were unfractionated DSIMs (lanes 1 and 2) and the CHAPS-insoluble fraction of DSIMs (lanes 3 and 4) before (lanes 1 and 3) and after (lanes 2 and 4) incubation.

detected three protein bands as expected (Figure 4B, lane 3). After incubation, the 20- and 24-kDa SCD fragments characteristic of SCDP cleavage were detected (Figure 4B, lane 5). A peripheral protein of the Golgi apparatus, Golgi 58 kDa (Bloom and Brashear, 1989), was present in DSIMs but not detected in salt-washed microsomes (Figure 4C), an indication that the 500 mM NaCl/15 mM EDTA washing procedure was effective. These results suggest that peripheral membrane proteins are not required for the proteolytic degradation of SCD described here.

We next asked whether SCD degradation requires lumenal proteins. Selective release of lumenal ER proteins by freeze–thawing or extraction with low concentrations of nonionic detergent did not affect SCD degradation. However, release of lumenal proteins by these methods is incomplete. We took advantage of our observations regarding the solubility of SCD to design a procedure that more completely solubilizes lumenal proteins without solubilizing SCD. In the experiment of Figure 5, DSIMs were incubated with [^{3}H]DFP to label the microsomal esterases. These are abundant soluble 60-kDa lumenal proteins (Ozols, 1989). Lumenal proteins were then extracted with 2% CHAPS. The CHAPS-insoluble material was separated by ultracentrifugation, solubilized with DOC/TX100, and incubated overnight. SCD was recovered with the CHAPS-insoluble material (Figure 5, lane 3). SCD was degraded, and the proteolytic fragments of SCD characteristic of SCDP cleavage were detected in the CHAPS-insoluble material after incubation (Figure 5, compare lanes 3 and 4). Autoradiography of the CHAPS-insoluble material showed only a trace of mi-

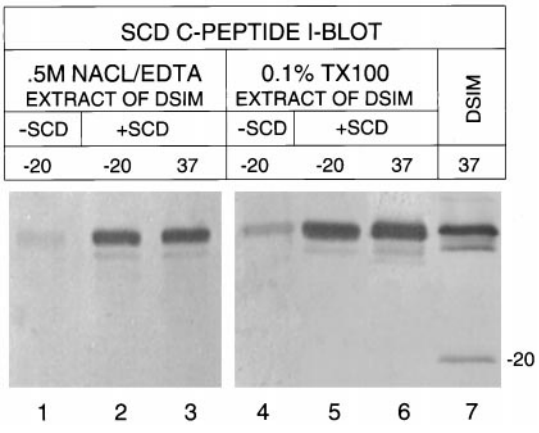

Figure 6. Immunoblot analysis of SCD degradation by peripheral, lumenal, and integral membrane protein preparations. Peripheral proteins and lumenal proteins of DSIMs were selectively extracted as described in MATERIALS AND METHODS. The peripheral protein preparation (lanes 1–3) and the lumenal preparation (lanes 4–6) were supplemented with purified SCD where indicated (lanes 2–3 and 5–6). Integral membrane proteins were extracted with 2% DOC/TX100 (lane 7). After incubation, the protein samples were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and probed with rabbit antiserum to a C-terminal peptide of SCD.

crosomal esterase (Figure 5B). These results suggest that lumenal proteins are not required for SCD degradation.

To confirm the results of the previous experiments, we examined preparations of peripheral proteins and lumenal proteins of DSIMs for proteolytic activity against SCD. Peripheral proteins were prepared by extracting DSIMs with 500 mM NaCl/15 mM EDTA. The peripheral protein preparation was then dialyzed to lower the NaCl concentration, and DOC/TX100 was added. Lumenal proteins were selectively extracted with 0.1% TX100. The lumenal protein preparation and the peripheral protein preparation contained only traces of SCD (Figure 6), an indication that the preparations were substantially free of integral membrane proteins. When supplemented with purified SCD and incubated overnight, neither preparation generated detectable quantities of the 20-kDa proteolytic fragment of SCD (Figure 6, lanes 3 and 6).

SCDP Cleaves SCD between Residues 176 and 177 in a Conserved Hydrophobic Segment

SCD was recovered in the TX100 extract of salt-washed DSIMs. SCD is one of a spectrum of microsomal proteins that are not solubilized by nonionic detergents from native microsomes at low ionic strength but are solubilizied by nonionic detergents from salt-washed microsomes. The 20-kDa fragment was generated by incubating the extract overnight at 6–8°C. Thus, SCDP was also solubilized under these conditions. SCD and the 20-kDa fragment were partially purified by passing the extract through a DEAE column. SCD and the 20-kDa fragment were recovered in the DEAE flow-through fractions (Figure 7). Sequence analysis of the 20-kDa fragment purified by SDS-

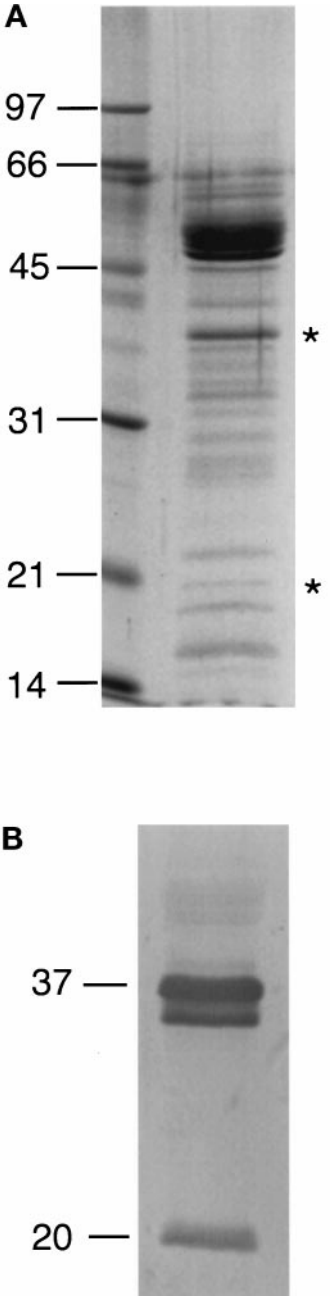

Figure 7. Isolation of the 20-kDa fragment of SCD. Solubilized DSIMs were incubated to generate the 20-kDa fragment. The preparation was passed over a DEAE-cellulose column. (A) Coomassie blue–stained SDS-PAGE gel of the DEAE flow-through fraction. (B) Immunoblot analysis of the DEAE flow-through fraction using antibody against the C terminus of SCD.

Table 1. N-terminal sequence analysis of the 20-kDa fragment

Cycle	Residue	Expected from cDNA	Position
1	Phe	Phe	177
2	Phe	Phe	178
3	X[a]	Ser	179
4	X[a]	His	180
5	Val	Val	181
6	Gly	Gly	182
7	Trp	Trp	183
8	Leu	Leu	184
9	Leu	Leu	185

[a] X, Not identified.

PAGE and isolated by electroelution revealed the sequence shown in Table 1.

DISCUSSION

Our data show that SCD is selectively degraded by a constitutive microsomal protease with properties of an integral membrane protein. Several observations support our view that the in vitro proteolytic system described here is responsible for the rapid turnover of desaturase observed in vivo. First, the degradation of SCD in isolated microsomes is highly selective. When DSIMs are incubated intact at 37°C, SCD is the only major microsomal protein significantly degraded after 12 h (Ozols, 1997). When DSIMs are incubated in the presence of detergent, one other protein, an unidentified 96-kDa protein, is also rapidly degraded. All other abundant microsomal proteins in DSIMs appear to be relatively resistant to proteolysis. These results correlate with seminal observations regarding the turnover of microsomal proteins in vivo. Microsomal proteins are degraded independently, and most microsomal proteins have half-lives of 2–6 d (Omura *et al.*, 1967; Arias *et al.*, 1969). Second, the rate of SCD degradation in vitro is identical to the rate observed in vivo. The half-life of SCD in vivo was determined by inhibiting protein synthesis during dietary induction of SCD and measuring desaturase activity in microsomes isolated from treated animals (Oshino and Sato, 1972). Under these conditions desaturase activity declines, with a half-life of 3–4 h. The half-life of SCD in detergent-solubilized DSIMs at 37°C is also 3–4 h. Third, our results suggest that the protease responsible for SCD degradation (SCDP) is a resident protein of the endoplasmic reticulum. Degradation proceeds in microsomal vesicles in the absence of ATP or cytosol, a condition that prevents membrane fusion events (Stafford and Bonifacino, 1991; Wikstrom and Lodish, 1992). If SCDP were confined to a non-ER component of microsomes, e.g., an endosomal or trans-Golgi compartment, it would probably not have access to SCD in vesicles derived from ER before solubilization. The fact that SCD is degraded in microsomal vesicles suggests that SCDP is in close proximity to SCD before vesiculation of the ER during homogenization. Several observations make unlikely the possibility that SCDP is a cytosolic protease or lysosomal protease artifactually adsorbed on the surface of microsomes. Incubation of cytosol with microsomes does not accelerate degradation. Extensive washing procedures to remove trapped cytosolic proteins do not diminish SCD degradation. Procedures that selectively remove extrinsic proteins from the cytosolic ER surface and soluble proteins from the ER lumen fail to inhibit SCD degradation. These results provide strong evidence that SCDP is an integral membrane protein or protein complex of the ER.

We have shown that purified SCD is degraded by normal rat liver microsomes and that this results in the generation of proteolytic SCD fragments similar to those produced by DSIMs. Therefore, it appears that SCDP is constitutively expressed rather than coinduced with SCD. This is consistent with the observation that desaturase is degraded in vivo at the same rate 5 and 16 h after dietary induction (Oshino and Sato, 1972). If the protease were coinduced, we would expect SCD to be degraded more rapidly later in the induction. Thus, degradation of SCD does not appear to require a molecular trigger to activate proteolysis. This contrasts with another target of ER degradation, 3-hydroxy-3-methylglutamyl-CoA (HMG-CoA) reductase. HMG-CoA reductase is a relatively stable enzyme under conditions of sterol depletion but is rapidly degraded in the presence of mevalonate (Brown and Goldstein, 1980). The protease that degrades HMG-CoA reductase is also constitutively expressed but differs from SCDP in its sensitivity to protease inhibitors. Degradation of HMG-CoA reductase is inhibited by cysteine protease inhibitors (Inoue *et al.*, 1991) and the proteosome inhibitor lacticystin (McGee *et al.*, 1996). Neither of these types of protease inhibitor affect SCD degradation (Ozols, 1997).

During degradation in vitro, proteolytic fragments of SCD with apparent molecular masses of 20 and 24 kDa accumulate. The 20-kDa fragment is recognized by antisera against a C-terminal peptide of SCD. The 24-kDa fragment is not recognized by the C-peptide antisera. These results suggest that during in vitro degradation SCD is cleaved near the middle of the polypeptide chain, generating a 24-kDa N-terminal fragment and a 20-kDa C-terminal fragment. This was confirmed by isolating the 20-kDa fragment and identifying its N terminus as Phe^{177}. The quantity of the 20-kDa fragment in the digests is never >1% of the original SCD starting material. Presumably this reflects the activity of the system for degrading the 20-kDa fragment. SCD is 358 amino acids in length and contains several hydrophobic domains (Thiede *et*

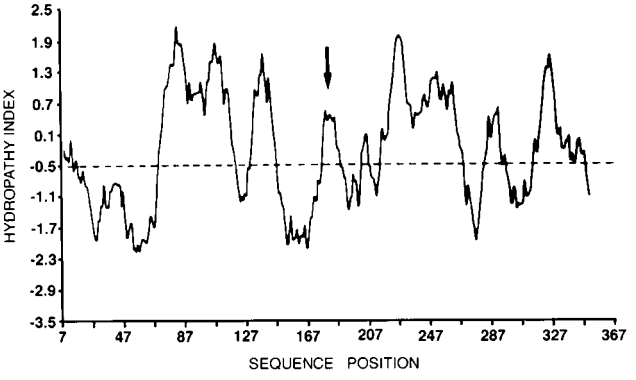

Figure 8. Hydropathy analysis of rat liver SCD. Hydropathy plot of the amino acid sequence (Thiede *et al.*, 1986) was analyzed by the method of Kyte and Doolittle (1982). The position of the identified cleavage site is indicated by an arrow.

al., 1986; Stukey *et al.*, 1990; Shanklin *et al.*, 1994). Catalytically essential histidine residues are clustered in three segments, designated region Ia (residues 114–133), region Ib (residues 149–169), and region II (residues 291–311) (Shanklin *et al.*, 1994). The Phe–Phe bond at residues 176–177 occurs in a 12-residue hydrophobic segment (Figure 8). The aromatic residues and the hydrophobicity of the segment are conserved in the δ-9 SCD protein of *Saccharomyces cerevisiae* (Stukey *et al.*, 1990). The conservation of this segment is consistent with the hypothesis that this segment may be critical to the proteolytic regulation of SCD. This hypothesis can be tested by site-directed mutagenesis. Although the topology of yeast SCD place residues 176–177 on the cytosolic side of the ER membrane, the hydrophobicity of the cleavage site segment suggests that it may interact with the membrane.

Protein degradation in the ER serves at least two functions: quality control and metabolic control (Fra and Sitia, 1993; Bonifacino and Klausner, 1994). Recent evidence suggests that cytosolic proteases are involved in the quality control function of the ER, i.e., the degradation of structurally abnormal proteins (reviewed in Kopito, 1997). The proteases that regulate critical metabolic functions of the ER by degrading key rate-limiting enzymes, e.g., SCD and HMG-CoA reductase, have not been identified. Degradation of purified SCD by solubilized microsomes provides a system that should facilitate the purification of the constitutive microsomal protease described here. The significance of the 20-kDa fragment detected during in vitro degradation remains to be determined.

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