Expression of the Mature and the Pro-Form of Human Sterol Carrier Protein 2 in *Escherichia coli* Alters Bacterial Lipids†

Janet E. Matsuura,‡ Henry J. George,‡ N. Ramachandran,‡ Juan G. Alvarez,§ Jerome F. Strauss, III,§ and Jeffrey T. Billheimer*,‡

Du Pont Merck Pharmaceutical Company, Experimental Station, Wilmington, Delaware 19880-0400, and Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Received September 2, 1992

ABSTRACT: Sterol carrier protein 2 (SCP_2) is a protein that is believed to be involved in the intracellular transport of cholesterol and phospholipids. Expression in mammalian COS cells of a cDNA encoding SCP_2 revealed that the mature protein is synthesized as a pro-form containing a 20-residue amino-terminal leader sequence. The function of this presequence is currently not known, and pro-SCP_2 is generally not detected in tissues. In order to obtain large quantities of pro-SCP_2 as well as the mature form of human SCP_2, *Escherichia coli* expression plasmids were constructed. Both proteins were produced in high yield (10–30% of the total cell protein) and were found in the supernatant fraction after cell lysis. Recombinant human SCP_2 and pro-SCP_2 were purified to homogeneity by acid precipitation followed by ion-exchange chromatography. Both recombinant human SCP_2 and pro-SCP_2 had sterol exchange activity similar to that seen with SCP_2 purified from rat liver. In addition, the lipid content of SCP_2- and pro-SCP_2-producing *E. coli* was analyzed. Acidic lipids were significantly increased in the transfected cells. Specifically, fatty acids were increased 2–3-fold, phosphatidylglycerol was increased 2-fold, and lipid A was increased 3–4-fold, while neutral lipids were decreased 2–3-fold as compared to control cells. This alteration of the lipid composition of *E. coli* expressing SCP_2 or pro-SCP_2 is consistent with the proposed role for SCP_2 in intracellular lipid movement.

Sterol carrier protein 2 (SCP_2)[1] is a 13.2-kDa basic protein, p*I* of 8.6, which was initially isolated from rat liver cytosol (Bloj & Zilversmit, 1977) but has since been detected in a number of tissues (Teerlink et al., 1984). In in vitro studies, SCP_2 has been shown to transfer both sterols and phospholipids between membranes, and it is thought that this protein may have a similar function within the cell (Billheimer & Reinhart, 1990). Isolation of the cDNA for rat, mouse, and human SCP_2 (Billheimer et al., 1990a; Ossendorp et al., 1990; Moncecchi et al., 1991; Yamamoto et al., 1991) has revealed that SCP_2 is synthesized as a 15.3-kDa pro-form, from which a 20 amino acid leader sequence is removed to produce the mature 13.2-kDa form of SCP_2. It has been suggested that the 15.3-kDa pro-form might be an inactive form of SCP_2 which requires cleavage to generate the active form.

The cellular location where cleavage occurs has yet to be determined, but both a peroxisomal location and a mitochondrial location have been suggested. The evidence for a peroxisomal location includes the immunolocalization of SCP_2 in peroxisomes (Van Amerongen et al., 1989; Keller et al., 1989; Tsuneoka et al., 1989) and the presence of a peroxisomal targeting sequence, Ala-Lys-Leu, in the C-terminus (Subramani, 1991). Additionally, in cells from subjects with Zellweger syndrome, which do not produce functional peroxisomes, the 15.3-kDa form of SCP_2 is produced, but then apparently degraded without yielding the mature 13.2-kDa form (Suzuki et al., 1990). However, it is relatively uncommon for peroxisomal proteins to undergo cleavage of N-terminal presequences during or after import into the peroxisomes (Subramani, 1991). By contrast, cleavage of N-terminal presequences regularly occurs for many mitochondrial proteins, and SCP_2 has been found to associate with mitochondria (Becker et al., 1992; Megli et al., 1985; McNamara et al., 1989, McLean et al., 1989). The 20 amino acid leader sequence has some similarity to known mitochondrial targeting sequences including a relative lack of acidic amino acids, an abundance of hydroxylated amino acids, and the ability to form an amphiphilic α-helical structure, but it is not enriched in basic amino acids (Nicholson & Neupert, 1988).

Studies addressing the physiological function of SCP_2 and its pro-form have been slowed by the limited availability of large amounts of pure SCP_2 and the relative lack of pro-SCP_2 found in tissue samples. In this paper, we report the production of milligram quantities of both the mature and the pro-form of human SCP_2 using an *Escherichia coli* expression system. Additionally, we have analyzed the impact of the overproduction of SCP_2 or pro-SCP_2 on the lipid profile of the *E. coli* expressing these proteins.

MATERIALS AND METHODS

E. coli Expression System. The pCMV5 expression vector, previously used to express human SCP_2 in COS cells (Yamamoto et al., 1990), was transformed into *E. coli* strain MC1009, and four isolated colonies were chosen for cesium chloride preparations of plasmid DNA (Sambrook et al., 1989). Oligonucleotide primers were designed for polymerase chain reaction (PCR) amplification and isolation of the DNA encoding either mature SCP_2 or pro-SCP_2. The 5′-primer for SCP_2 was CTTAAGCTTACATATGGCTTCTGCAAGT-GATGGATTTAAGC, and that for pro-SCP_2 was CT-TAAGCTTACATATGGGTTTTCCGGAAGCGC (the un-

† This work was supported in part by NIH Grant HD-06274.

* Address correspondence to this author at Du Pont Merck Pharmaceutical Co., P.O. Box 80400, Wilmington, DE 19880-0400. Telephone: (302) 695-7103. FAX: (302) 695-7054.

‡ Du Pont Merck Pharmaceutical Co.

§ University of Pennsylvania School of Medicine.

[1] Abbreviations: SCP_2, sterol carrier protein 2; pro-SCP_2, pro-form of sterol carrier protein 2 containing an additional 20-residue amino-terminal sequence; PCR, polymerase chain reaction; IPTG, isopropyl β-D-thiogalactopyranoside.

0006-2960/93/0432-567$04.00/0

derlined bases are homologous to the protein's DNA sequence). The same 3′ primer was used for the expression of both proteins: CTTTCCATGGATCCTTATCAGAGCTTAGCGTTGCCTG. The 5′ primers added a *Nde*I restriction site directly 5′ of the desired sequence, and this site provided the ATG initiation codon for SCP_2. Additionally, a serine to alanine change for the first amino acid of SCP_2 was engineered to maximize initiation of translation and to ensure cleavage of the initial methionine residue by *E. coli* methionylaminopeptidase (Hirel et al., 1989). The 3′ primer included both the TGA and the TAA translational termination signals followed by unique restriction sites for *Bam*HI and *Nco*I. The reaction mixture for PCR amplification of SCP_2 or pro-SCP_2 included 1 μg of the appropriate 5′- and 3′-oligonucleotide primer combination, 0.1 ng of the pCMV5 DNA template, and the four deoxynucleotides at a final concentration of 50 μM/dNTP in a 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$ buffer, pH 8.3. A total of 29 repetitive cycles of 1 min at 94 °C, 1 min at 58 °C, and 1 minute at 72 °C, followed by a 7-min period at 72 °C to allow for the completion of synthesis, were conducted. The reaction mixture was then transferred to a new tube, and the DNA was purified using the GENECLEAN kit (BIO 101 Inc., La Jolla, CA). The production of a single PCR product of the appropriate size was confirmed for each sample by applying an aliquot of the DNA to a 1% TAE (Tris–acetate/EDTA buffer)–agarose gel and viewing the ethidium bromide-stained gel under ultraviolet light.

The PCR-amplified DNA was then digested with *Nde*I and *Bam*HI, and the reisolated fragment was ligated into a pTAC-based expression plasmid, which had been digested with the same restriction enzymes and transformed into *E. coli* strain NF1829 using standard techniques (Sambrook et al., 1989). Cells containing the plasmid were selected, and the plasmid DNA was reisolated and subjected to restriction analysis to confirm the presence of the DNA insert encoding SCP_2 (pHG240/4) or Pro-SCP_2 (pJM104/6).

Production of SCP_2 and Pro-SCP_2. *E. coli* strains, W3110 or W3110F′, were transformed with the plasmid containing the DNA fragment encoding either SCP_2 (pHG240/4) or pro-SCP_2 (pJM104/6), respectively. Both plasmids contain a modified pTac promoter/operator system, the β-lactamase gene encoding ampicillin resistance, an optimized ribosomal binding sequence, a tl3 transcriptional terminator, atpE epsilon translational enhancer sequences, REP1 mRNA stabilization sequences, and a ColE1 (rop-) replication origin. The plasmid for the expression of SCP_2 also contained the parB (hok-sok) plasmid stabilization loci and a laci repressor gene cassette.

E. coli expressing SCP_2 or pro-SCP_2 were produced by inoculating 1 L of LB media plus ampicillin (100 μg/mL) with 4 mL of overnight culture of cells. The cells were grown at 37 °C with shaking until they reached an A_{600} of 0.5, induced by the addition of 100 mM isopropyl β-D-thiogalactopyranoside (IPTG) at a final concentration of 0.5 mM and incubated for an additional 3–4 h. The cells were harvested by centrifugation, resuspended in 10 mM Tris-HCl/5 mM EDTA/10% glycerol, pH 8.0, and stored at −70 °C until protein purification was conducted.

Purification of SCP_2 and Pro-SCP_2. Cell suspensions were thawed at room temperature, and the cells were repelleted and resuspended in a 0.1 potassium phosphate buffer, pH 6.8, containing 0.5 mM EDTA/1 mM dithiothreitol (KPED buffer), containing 1 mg/L leupeptin. The cells were ruptured by multiple passes through a Ranne homogenizer at 10 000 psi or by sonication, and supernatants were collected by centrifugation at 4000*g* for 30 min at 4 °C. The remainder of the purification procedure was conducted at 4 °C. The pH of the supernatants was lowered to pH 5.2 using 6 N HCl. After 15 min of mixing, the resulting precipitates were pelleted by centrifuging at 24000*g* for 20 min, and the pH of the acid-treated supernatants was increased to pH 7.4 by the addition of 8 M KOH. As for the acid treatment, a 15-min period of mixing followed by centrifugation was conducted to collect the supernatants. The supernatants were dialyzed against 10 mM KPED buffer and separately applied to a 10-mL Mono-S column equilibrated with 10 mM KPED buffer. The column was washed extensively to remove any nonabsorbed proteins and the SCP_2 or pro-SCP_2 eluted with 80 mL of a 0.06 M KCl linear gradient. The fractions comprising the protein peak were pooled and dialyzed against water or buffer and lyophilized. The proteins were shown to be homogeneous by SDS–PAGE electrophoresis. The identities of the proteins were further confirmed by Western blotting and by N-terminal amino acid sequencing of the first 15–20 amino acids using a Porton Instruments PI 2090E sequencer (Porton Instruments, Tarzana, CA).

Sterol Transfer Activity. The ability of the purified SCP_2 and pro-SCP_2 to transfer cholesterol between donor and acceptor membranes was assayed as previously described (Billheimer et al., 1990b). The assay measures the ability of a protein to facilitate the movement of [^{14}C]cholesterol from [^{14}C]cholesterol/egg yolk phosphatidylcholine liposomes to heat-treated rat liver mitochondria. Rat liver SCP_2, which was isolated and purified as previously described, was assayed concurrently with the purified human SCP_2 and pro-SCP_2 samples (Morris et al., 1988). Protein was assayed by the method of Bradford (1976).

Analysis of the Lipid Content of E. coli Expressing SCP_2 or Pro-SCP_2. One-liter cultures of *E. coli* expressing either SCP_2, pro-SCP_2, or an unrelated protein, a 43-kDa fusion protein consisting of a 30-kDa outer membrane protein from *Brucella abortus*, BCS30 (Mayfield et al., 1988), and pro-interleukin-1β, were grown up as above, except that M9 minimal medium was substituted for the LB media. The lipids were extracted and analyzed by the method of Alvarez and Touchstone (1992). Briefly, the cell pellets were extracted twice with 20 volumes of chloroform/methanol/water (10:10:1), and the combined extracts were evaporated to dryness, and the residue was redissolved in chloroform. The samples were applied to an aminopropyl column (Worldwide Monitoring, Horsham, PA), equilibrated with hexane, and four fractions were eluted: neutral lipids, fatty acids, neutral phospholipids and glycolipids, and acidic phospholipids and glycolipids. Each fraction was subsequently analyzed by thin-layer chromatography on silica gel HP-K plates (Whatman, Clifton, NJ), and following $CuSO_4$ staining, the samples were quantitated by scanning densitometry. The values reported are the mean of samples from three plates.

RESULTS

Following IPTG induction, 20–30% of the total cell protein of cells containing the expression vector for SCP_2, pHG240/4, was recombinant SCP_2 (Figure 1). All of the recombinant protein could be isolated from the soluble fraction after cell lysis. The majority of the recombinant pro-SCP_2 produced in cells containing the expression vector for pro-SCP_2, pJM104/6, was also found in the soluble fraction. However, detectable amounts of the protein remained with the pellet following centrifugation of cell lysates, suggesting that some of this material may form inclusion bodies. The level of

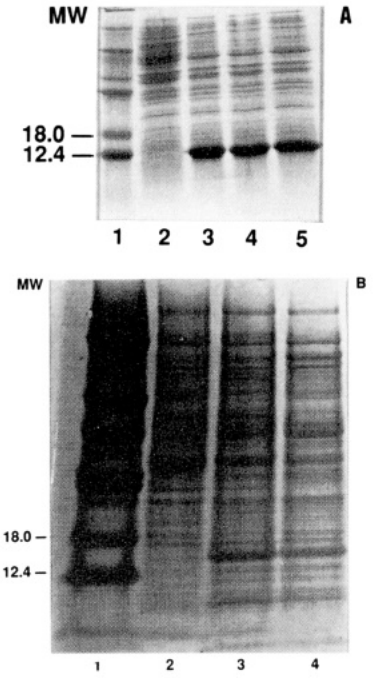

FIGURE 1: SDS–PAGE gels of *E. coli* expressing human SCP_2 (gel A) or pro-SCP_2 (gel B). Molecular mass markers (lane 1) used were 92, 55, 43, 36, 29, 18, and 12.4 kDa. Gel A is a 15% polyacrylamide gel stained with Coomassie blue. Lanes 3–5 are preparations of total cell lysates from SCP_2-expressing *E. coli* clones, HG240/1, HG240/2, and HG240/3, induced with IPTG. Lane 2 shows total cell lysate from the uninduced clone HG240/1. Gel B is a 10–20% Tricine–SDS gel stained with Coomassie blue. Lane 1 contains molecular mass markers; lane 2, total cell lysate of control *E. coli* (cells which were not transfected with the DNA for pro-SCP_2) following induction with IPTG; lane 3, total lysate of cells expressing pro-SCP_2 following induction with IPTG; and lane 4, cytosolic fraction of cells expressing pro-SCP_2 following induction with IPTG.

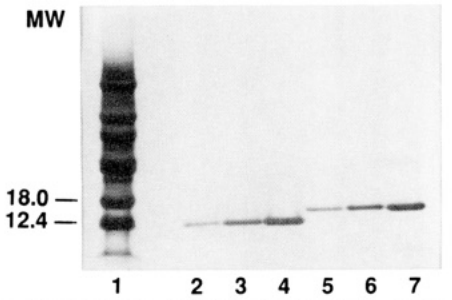

FIGURE 2: Homogeneity of SCP_2 and pro-SCP_2 purified from *E. coli*. Aliquots of human SCP_2 (lanes 2–4) and pro-SCP_2 (lanes 5–7) at increasing amounts of protein (1, 3, and 7 μg) were subjected to SDS–PAGE electrophoresis on a 10–20% Tricine–SDS gel and stained with Coomassie blue. The molecular mass standards (lane 1) are as described in Figure 1.

expression of pro-SCP_2 after induction with IPTG was approximately 10–20% of the total cell protein. The lower level of production of pro-SCP_2 may reflect commonly seen differences between batch preparations, or may reflect a lower level of production of the longer pro-form of the protein by *E. coli*.

Preparations of SCP_2 and pro-SCP_2 were purified to homogeneity from 1-L cultures of *E. coli*. When aliquots of each purified preparation were applied to 10–20% Tricine/SDS gels, a protein band migrating with a molecular mass of 12 500 Da was seen for the SCP_2 preparation, and a protein band of 15 000 Da was seen for the pro-SCP_2 preparation (Figure 2). At the largest amount of protein, higher molecular mass bands corresponding to the dimers for SCP_2 and for pro-SCP_2 were evident. A yield of 30–40 mg/L was typical for cultures of *E. coli* expressing SCP_2, while 3–5 mg/L was more common for our *E. coli* expression system for pro-SCP_2.

Table I: Sequence of the N-Terminal Amino Acids for *E. coli*-Expressed Human SCP_2 and Pro-SCP_2

	-20			1			20
SCP_2[a]	MGFPEAASSF	RTHQIEAVPT		SSASDGFKAN	LVFKEIEKKL		
SCP_2 (*E. coli*)				ASASDGFKAN	LVFKE		
pro-SCP_2 (*E. coli*)	GFPEAASSF	RTHQIEAVPT		SS			

[a] Protein sequence derived from the cDNA sequence for human SCP_2 (Yamamoto et al., 1991).

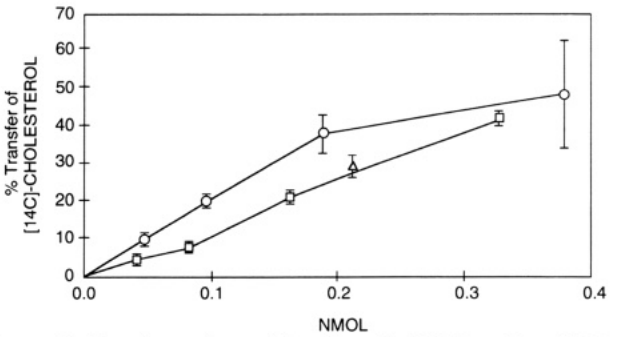

FIGURE 3: Sterol transfer activity of purified SCP_2 and pro-SCP_2. Aliquots of the purified proteins were incubated for 20 min at 37 °C with [^{14}C]cholesterol/egg yolk phosphatidylcholine liposomes and heat-treated mitochondria. At the end of this incubation period, the percentage transfer of label was measured for human SCP_2 (○) and for human pro-SCP_2 (□). SCP_2 isolated from rat liver (△) at a single protein amount was assayed concurrently. All values shown are the mean ± SD for triplicate samples.

Sequencing of the N-terminal amino acids confirmed that the proteins isolated from the *E. coli* had the expected sequence (Table I). The N-terminal amino acid sequence for the SCP_2 protein expressed by *E. coli* was identical to that predicted from the cDNA (Yamamoto et al., 1991) with the exception of the first amino acid, which was an alanine instead of a serine. However, this amino acid change was engineered into the design of the original PCR primer to promote efficient translation and cleavage of the N-terminal methionine and was therefore expected. The N-terminal amino acid sequence for the pro-SCP_2 expressed in *E. coli* was identical to that predicted from the cDNA with processing of the N-terminal methionine residue.

To ascertain if the *E. coli*-expressed proteins possessed lipid transfer activity, their ability to promote movement of cholesterol between membranes was assayed. The nanomoles of purified SCP_2 or pro-SCP_2 was plotted against the percentage of [^{14}C]cholesterol transferred from [^{14}C]cholesterol/egg yolk phosphatidylcholine liposomes to heat-treated mitochondria (Figure 3). Both the mature and the pro-form of SCP_2 stimulated the transfer of cholesterol from donor to acceptor membranes. The levels of activity for the expressed proteins were similar to those obtained with SCP_2 purified from rat liver.

Although prokaryotic organisms such as *E. coli* do not contain sterols, they do have a complement of phospholipids and other lipid species in their membranes which could potentially be subject to SCP_2-mediated transfer (Billheimer & Reinhart, 1990). Analysis of the lipid profile of *E. coli* transfected with the DNA from human SCP_2 or pro-SCP_2 revealed substantial differences from that seen for non-plasmid-containing cells. Four lipid fractions were eluted from an aminopropyl column and analyzed. The first three fractions (neutral lipids, fatty acids, and neutral phospholipids and glycolipids) are shown in Figure 4. Scanning densitometry

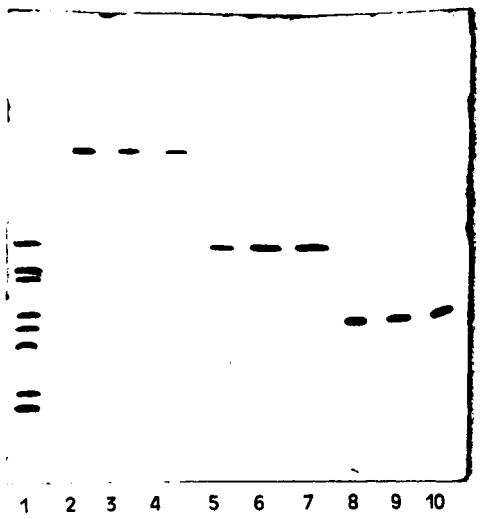

FIGURE 4: $CuSO_4$-stained HPTLC chromatogram of *E. coli* lipids eluted in fractions 1–3 from the aminopropyl column. Each group of three samples are lipids extracted from control *E. coli* which do not express SCP_2 or pro-SCP_2 from *E. coli* which express SCP_2, and from *E. coli* which express pro-SCP_2, respectively. Lane 1 contains lipid standards: oleic acid, phosphatidylglycerol, cardiolipin, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, and sphingomyelin. Lanes 2–4 contain the nonpolar lipid fractions, lanes 5–7 contain the fatty acid fractions, and lanes 8–10 contain the neutral phospholipid and glycolipid fractions.

suggests that there was a 2-fold and a 3-fold decrease in the nonpolar lipid fraction for the SCP_2- and the pro-SCP_2-transfected cells as compared to host cells. Fatty acids, however, increased 2-fold and 2.5-fold in the SCP_2- and pro-SCP_2-transfected cells over levels seen for control cells. The increase in fatty acid content appears to be a generalized increase in all fatty acids and not an increase in an individual fatty acid, as analysis of individual fatty acid species by diphasic two-dimensional HPTLC–fluorescence spectrodensitometry revealed no differences in the percentages of individual species (data not shown). No differences were seen for phosphatidylethanolamine levels between control and transfected cells. In initial experiments, the lipids from *E. coli* expressing an unrelated protein, a 43-kDa fusion protein of BCSP31 (Mayfield et al., 1988) and pro-interleukin-1β, were also analyzed. The fusion protein was expressed at a level of 10–20% of the total cellular protein. The lipid composition of these bacteria was found to be identical to that of the nontransfected control cells.

The fourth lipid fraction analyzed, the acidic glycolipids and phospholipids, is shown in Figure 5. As compared to control cells, the SCP_2- and pro-SCP_2-transfected cells showed a 2-fold increase in phosphatidylglycerol and a 3-fold and 4.5-fold increase in a lipid species, which migrates like *E. coli* lipid A. Lipid A is a major component of *E. coli* outer membranes and serves as the hydrophobic anchor for lipopolysaccharide (Raetz et al., 1991). The molecule is a disaccharide of glucosamine, which is phosphorylated and acylated. The acidic lipid species, which was increased in our transfected cells and comigrated with authenic lipid A, also gave positive reactions for orcinol and molydenum staining, which demonstrate the presence of sugar hemiketals and phosphate moieties, respectively. Furthermore, hydrolysis with 0.1 N NaOH resulted in the release of fatty acid and a slower migrating component (Figure 5B).

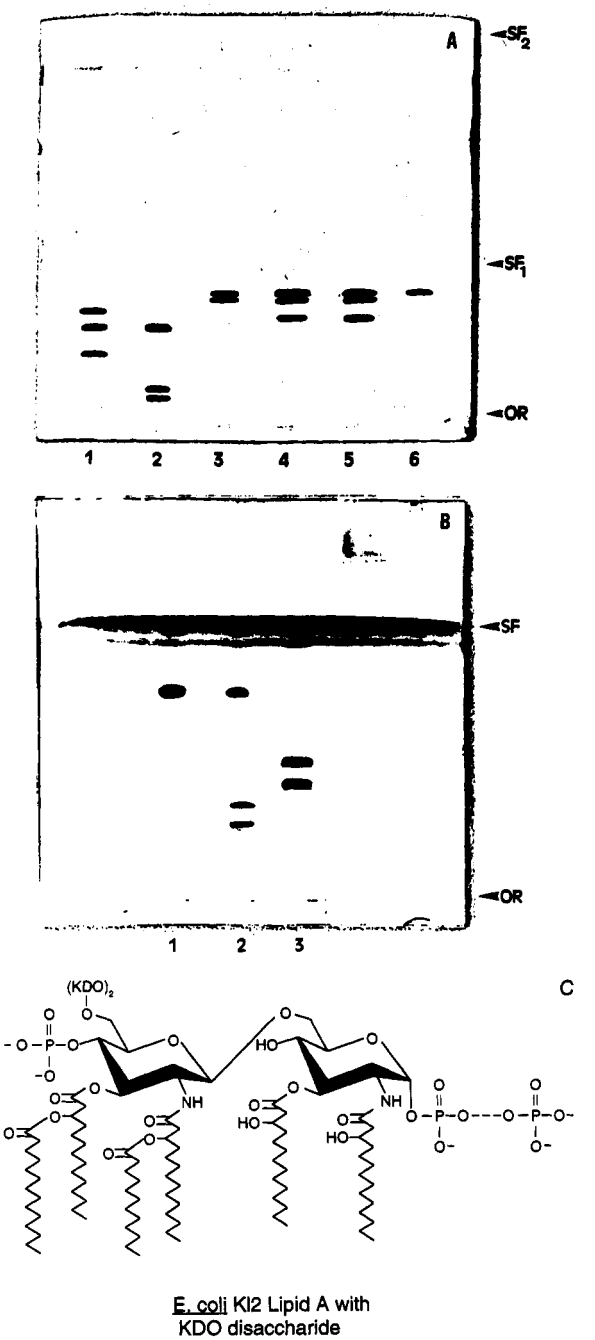

FIGURE 5: Analysis of the acidic lipids from *E. coli*. The locations of the origin (OR) and the solvent front (SF) or fronts (SF_1 and SF_2) are indicated for each plate. Panel A shows the $CuSO_4$-stained HPTLC chromatogram of the acidic polar lipids, fraction 4 from the aminopropyl column. *E. coli* lipid A standard was applied to lane 1, and phospholipid standards (phosphatidylethanolamine, phosphatidylcholine, and sphingomyelin) were applied to lane 2. Lanes 3–5 contain the phosphatidylglycerol fraction from control, SCP_2-expressing, and pro-SCP_2-expressing *E. coli*. Lane 6 contains a phosphatidylglycerol standard. Panel B shows the $CuSO_4$-stained HPTLC chromatogram of alkaline hydrolysis-treated lipid isolated from SCP_2-transfected *E. coli*, which migrated like lipid A in panel A. Lane 1 contains fatty acid standard, lane 2 contains the lipid A-like sample obtained from SCP_2-transfected *E. coli* after alkaline hydrolysis, and lane 3 contains lipid A standard. Panel C shows the structure of *E. coli* lipid A with 3-deoxy-D-mannooctulosonic acid (KDO) disaccharide [derived from Raetz et al. (1991)].

DISCUSSION

There is considerable interest in factors that regulate intracellular lipid trafficking. SCP_2 has received attention in this regard because it is highly conserved among species with greater than 85% homology between the amino acid sequences of human, mouse, rat, and bovine SCP_2 (Yamamoto et al., 1991; Moncecchi et al., 1991; Pastuszyn et al., 1987; Westerman & Wirtz, 1985), it facilitates the movement of sterols and phospholipids between membranes in in vitro assays

(Billheimer et al., 1990b; Crain & Zilversmit, 1980), and it has also been detected in all tissues studied (Teerlink et al., 1984). These properties would be expected of a protein involved in a fundamental process in lipid metabolism. Nonetheless, a number of proteins have been suggested as potentially facilitating cholesterol movement between membranes, but most of them have since been found to serve other functions (Habig et al., 1974; Gordon et al., 1983; Szeigolet, 1984).

Progress in defining the physiological role of SCP_2 has been hampered by the limited amounts of the protein which can be isolated from tissue. Advances in molecular cloning techniques and the isolation of the cDNA for the protein (Yamamoto et al., 1991) have made it possible to produce human SCP_2 and its pro-form in milligram quantities from *E. coli*. Our yield of 30 mg/L of *E. coli* culture for SCP_2 is equivalent to the amount of proteins which could be isolated from the livers of several hundred rats using a similar purification protocol (Morris et al., 1988). Moncecchi et al. (1991) have also used an *E. coli* expression system to produce mouse SCP_2, but were only able to purify 110 μg of the protein/L of cell culture. While it is generally not possible to isolate the pro-form of SCP_2 from tissue, we were able to produce 3 mg of human pro-SCP_2/L of culture using an *E. coli* expression system; Ossendorp et al. (1992) have recently reported a similar level of expression of the pro-form of rat liver SCP_2 using an *E. coli* expression system (6–7 mg/L).

As expected, the human SCP_2 produced in *E. coli* stimulated the transfer of cholesterol between membranes with an activity equal to, if not greater than, that observed with SCP_2 purified from rat liver. It was of note that human pro-SCP_2 was also capable of stimulating an increase in the rate of cholesterol transfer which was comparable to that seen for SCP_2 purified from rat liver. The pro-form of rat SCP_2 has also been shown to transfer phospholipid at an identical rate as the mature form of the protein (Ossendorp et al., 1992). Thus, cleavage of the presequence does not appear to be necessary for either sterol or phospholipid transfer activity. While pro-SCP_2 is clearly not an inactive precursor requiring cleavage to generate active SCP_2, the possible role of the 20 amino acid presequence as a targeting signal, particularly for mitochondrial targeting, has yet to be tested.

Production of large amounts of SCP_2 or pro-SCP_2 by *E. coli* was also found to alter the bacterial cells' lipid content. Specifically, the amounts of acidic lipids such as fatty acids, phosphatidylglycerol, and lipid A were increased. Currently, it is not known how the presence of these proteins brings about the observed increases in acidic lipids. However, it does not appear to be a result of the process of transfection and expression of a foreign protein as the lipid profile for *E. coli* expressing an unrelated protein was the same as that seen for nontransfected host cells. Perhaps, a charge interaction between the acidic lipids and the basic SCP_2 or pro-SCP_2 proteins removes regulatory pools of lipids which might otherwise provide feedback inhibition on their own synthetic pathways. Interestingly, lipid A, a major component of the *E. coli* outer membrane, is synthesized in part by proteins associated with the inner surface of the inner membrane (Raetz et al., 1991). One could speculate that SCP_2 may facilitate the movement of this lipid from the inner membrane through the periplasmic space and the peptidoglycan layer to its final destination in the outer membrane.

In summary, we report the expression of both the mature and the precursor forms of SCP_2 in *E. coli* in quantities of milligrams per liter of cell culture. Both the *E. coli*-expressed mature and precursor forms of SCP_2 efficiently transferred cholesterol between donor and acceptor membranes at a rate similar to SCP_2 isolated from rat liver, indicating that cleavage of the presequence was not a requirement for this activity. Overproduction of SCP_2 or pro-SCP_2 in *E. coli* resulted in an increase in acidic lipids in the cells. Use of these expression systems will allow for future studies addressing the role of the presequence in directing the protein to specific cellular locations and in determining the structure of SCP_2 by 3D NMR.

ACKNOWLEDGMENT

We thank Dr. Ramnath Seetharam for the N-terminal amino acid sequencing of SCP_2 and pro-SCP_2 and Donna Pedicord, Rosanne Stevenson, and Alden Huffman for technical assistance.

REFERENCES

Alvarez, J. G., & Touchstone, J. C. (1992) *J. Chromatogr. 577*, 142–145.

Becker, K., Guiard, B., Rassow, J., Söllner, T., & Pfanner, N. (1992) *J. Biol. Chem. 267*, 5637–5643.

Billheimer, J. T., & Gaylor, J. L. (1990) *Biochim. Biophys. Acta 1046*, 136–143.

Billheimer, J. T., & Reinhart, M. P. (1990) *Subcell. Biochem. 16*, 301–331.

Billheimer, J. T., Strehl, L. L., Davis, G. L., Strauss, J. F., III, & Davis, L. G. (1990) *DNA 9*, 159–163.

Bloj, B., & Zilversmit, D. B. (1977) *J. Biol. Chem. 252*, 1613–1619.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Crain, R. C., & Zilversmit, D. B. (1980) *Biochemistry 19*, 1433–1439.

Gordon, J. I., Alpers, D. H., Ockner, R. K., & Strauss, A. W. (1983) *J. Biol. Chem. 258*, 3356–3363.

Habig, W. H., Pabst, M. J., Fleischner, G., Gatmaitan, Z., Arias, I. M., & Jakoby, W. B. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3879–3882.

Hirel, PH.-H., Schmitter, J.-M., Dessen, P., Fayat, G., & Blanquet, S. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 8247–8251.

Keller, G. A., Scallen, T. J., Clarke, D., Maher, P. A., Krisans, S. K., & Singer, S. J. (1989) *J. Cell Biol. 108*, 1353–1361.

Mayfield, J. E., Bricker, B. J., Godfrey, H., Crosby, R. M., Knight, D. J., Halling, S. M., Balinsky, D., & Tabatabai, L. B. (1988) *Gene 63*, 1–9.

McLean, M. P., Puryear, T. K., Kahn, I., Azhar, S., Billheimer, J. T., Orly, J., & Gibori, G. (1989) *Endocrinology 125*, 1337–1344.

McNamara, B. C., & Jefcoate, C. R. (1989) *Arch. Biochem. Biophys. 275*, 53–62.

Megli, F. M., DeLisi, A., van Amerongen, A., Wirtz, K. W. A., & Quagliariello, E. (1986) *Biochim. Biophys. Acta 861*, 463–470.

Moncecchi, D., Pastuszyn, A., & Scallen, T. J. (1991) *J. Biol. Chem. 266*, 9885–9892.

Morris, H. R., Larsen, B. S., & Billheimer, J. T. (1988) *Biochem. Biophys. Res. Commun. 154*, 476–482.

Nicholson, D. W., & Neupert, W. (1988) in *Protein Transfer and Organelle Biogenesis* (Das, R. C., & Robbins, P. W., Eds.) pp 686–694, Academic Press, Boston.

Ossendorp, B. C., van Heusden, G. P. H., & Wirtz, K. W. A. (1990) *Biochem. Biophys. Res. Commun. 168*, 631–636.

Ossendorp, B. C., Geijtenbeek, T. B. H., & Wirtz, K. W. A. (1992) *FEBS Lett. 296*, 179–183.

Pastuszyn, A., Noland, B. J., Bazan, J. F., Fletterick, R. J., & Scallen, T. J. (1987) *J. Biol. Chem. 262*, 13219–13227.

Raetz, C. R. H., Ulevitch, R. J., Wright, S. D., Sibley, C. H., Ding, A., & Nathan, C. F. (1991) *FASEB J. 5*, 2652–2660.

Sambrook, J., Fritsch, E. F., & Maniatis, T., (1989) in *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Subramani, S. (1991) *Curr. Sci. 61*, 28–32.

Suzuki, Y., Yamaguchi, S., Orii, T., Tsuneoka, M., & Tashiro, Y. (1990) *Cell Struct. Funct. 15*, 301–308.

Szeigoleit, A. (1984) *Biochem. J. 219*, 735–742.

Teerlink, t., van der Krift, T. P., van Heusden, G. P. H., & Wirtz, K. W. A. (1984) *Biochim. Biophys. Acta 793*, 251–259.

Tsuneoka, M., Yamamoto, A., Fujiki, T., & Tashiro, Y. (1988) *J. Biochem. 104*, 560–564.

Van Amerongen, A., van Noort, M., van Beckhoven, J. R. C. M., Rommerts, F. F. G., Orly, J., & Wirtz, K. W. A. (1989) *Biochim. Biophys. Acta 1001*, 243–248.

Westerman, J., & Wirtz, K. W. A. (1985) *Biochem. Biophys. Res. Commun. 127*, 333–338.

Yamamoto, R., Kallen, C. B., Babalola, G. O., Rennert, H., Billheimer, J. T., & Strauss, J. F., III (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 463–467.