Molecular Characterization of Membrane-Associated Soluble Serine Palmitoyltransferases from *Sphingobacterium multivorum* and *Bdellovibrio stolpii*†

Hiroko Ikushiro,1* Mohammad Mainul Islam,1† Hiromasa Tojo,2 and Hideyuki Hayashi1*

Department of Biochemistry, Osaka Medical College, Takatsuki, Osaka 569-8686, Japan,1 and Department of Biochemistry and Molecular Biology, Osaka University, Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan2

Received 5 February 2007/Accepted 10 May 2007

Serine palmitoyltransferase (SPT) is a key enzyme in sphingolipid biosynthesis and catalyzes the decarboxylative condensation of L-serine and palmitoyl coenzyme A (CoA) to form 3-ketodihydrosphingosine (KDS). Eukaryotic SPTs comprise tightly membrane-associated heterodimers belonging to the pyridoxal 5′-phosphate (PLP)-dependent α-ketoglutarate synthase family. *Sphingomonas paucimobilis*, a sphingolipid-containing bacterium, contains an abundant water-soluble homodimeric SPT of the same family (H. Ikushiro et al., J. Biol. Chem. 276:18249–18256, 2001). This enzyme is suitable for the detailed mechanistic studies of SPT, although single crystals appropriate for high-resolution crystallography have not yet been obtained. We have now isolated three novel SPT genes from *Sphingobacterium multivorum*, *Sphingobacterium spiritivorum*, and *Bdellovibrio stolpii*, respectively. Each gene product exhibits an ∼30% sequence identity to both eukaryotic subunits, and the putative catalytic amino acid residues are conserved. All bacterial SPTs were successfully overproduced in *Escherichia coli* and purified as water-soluble active homodimers. The spectroscopic properties of the purified SPTs are characteristic of PLP-dependent enzymes. The KDS formation by the bacterial SPTs was confirmed by high-performance liquid chromatography/mass spectrometry. The *Sphingobacterium* SPTs obeyed normal steady-state ordered Bi-Bi kinetics, while the *Bdellovibrio* SPT underwent a remarkable substrate inhibition at palmitoyl CoA concentrations higher than 100 μM, as does the eukaryotic enzyme. Immunoelectron microscopy showed that unlike the cytosolic *Sphingomonas* SPT, *S. multivorum* and *Bdellovibrio* SPTs were bound to the inner membrane of cells as peripheral membrane proteins, indicating that these enzymes can be a prokaryotic model mimicking the membrane-associated eukaryotic SPT.

Sphingolipids are ubiquitous membrane components of the eukaryotic plasma membrane (32) and are known to be essential lipidic signaling molecules required for various cellular events, such as proliferation, differentiation, and apoptosis (22, 38, 54). In addition, sphingolipids together with cholesterol are the major components of the membrane microdomains called “lipid rafts,” which serve as platforms for signal transduction or the transport of various bioactive molecules via membrane trafficking (14, 25, 52).

Serine palmitoyltransferase (SPT) (EC 2.3.1.50) catalyzes the pyridoxal 5′-phosphate (PLP)-dependent condensation reaction of L-serine with palmitoyl coenzyme A (CoA) to generate 3-ketodihydrosphingosine (KDS). This reaction is the first committed step in the de novo biosynthetic pathway of all sphingolipids, producing long-chain bases (LCBs), the backbone structure of sphingolipids. SPT is thought to be the key enzyme regulating the cellular sphingolipid content (21). Eukaryotic SPTs are enriched in the endoplasmic reticulum, with their catalytic sites facing the cytosol (36), and function as heterodimers comprising two tightly membrane-bound subunits, called LCB1 and LCB2, which share a sequence similarity (∼25% identity) (10, 19, 20, 41, 42, 64). Recently a new subunit protein of the human SPT, SPTLC3, was found (24). Due to the high sequence similarity (68% identity) between SPTLC2 (LCB2 subunit of human SPT) and SPTLC3, SPTLC3 is thought to form a dimer with SPTLC1. LCB2 (SPTLC2) and SPTLC3 are the putative catalytic subunits carrying a lysine residue that forms the Schiff base with PLP. In contrast, LCB1 does not have such a motif (10, 19) and does not seem to function as the catalytic center. Nevertheless, LCB1 is regarded to be essential for the catalytic action of SPT (20), and mutations in the LCB1 gene are known to cause human hereditary sensory neuropathy type I (HSN1) (6, 11, 61). The roles of SPT activity in the pathogenesis of HSN1, however, are elusive at present (7, 17, 40). Elucidation of the structure-activity relationship of SPT is essential for understanding the role of the rate-limiting enzyme, SPT, in regulating the cellular sphingolipid homeostasis and for clarifying the underlying causes of HSN1. There is, however, little structural and mechanistic information on the mammalian SPT currently available, because the instability and the hydrophobic nature of each subunit have hindered the successful purification of recombinant SPT for crystallization and structural analysis (26).

Previously we found and isolated a water-soluble homodimeric SPT from *Sphingomonas paucimobilis* EY2395 (27). The *Sphingomonas* enzyme was successfully overproduced in *Escherichia coli* (27, 28). This bacterial prototype of the eukaryotic SPT provided a simple model system for study...
ing the enzyme reaction without detergent micelles or lipid membranes. However, despite the successful elucidation of the enzymological properties of the Sphingomonas SPT (29), we were unable to obtain crystals appropriate for a high-resolution X-ray analysis, which is essential for further clarification of the detailed catalytic mechanism of the enzyme. Therefore, we searched for SPT proteins that are suitable for crystallization in other sphingolipid-containing bacteria. One such candidate for the enzyme source is the genus Sphingobacterium, which is a deep-orange-pigmented rod belonging to the class Sphingobacteria of the phylum Bacteroidetes and is isolated from the environment (51) or from patients with opportunistic infections (8, 16, 23, 37, 60). Sphingobacterium has a high concentration of sphingophospholipids with unique branched LCBs, including ceramide phosphorylthanolamines, ceramide phosphorl-mycolinositol, and ceramide phospholipid-1-β-mannose as the major components (43, 44, 71, 72). Another candidate, Bdellovibrio, is a small curved rod belonging to the delta subclass of the phylum Proteobacteria that can be found in diverse environments, such as marine and fresh waters, sewage, and soil (47). Bdellovibrio is characterized by the unique predatory behavior by which it invades various other larger gram-negative bacteria and grows as a parasite in the intraperiplasmic space of the prey (46, 47, 56, 57). Bdellovibrio contains a phosphono ceramide, which carries the characteristic head group 1-hydroxy-2-aminoethyl phosphoryl (62). The bacteria listed above are exceptions in gram-negative bacteria in that they lack lipopolysaccharides and instead contain a large amount of sphingolipids, including glyosphingolipids (33, 67–70, 72); most gram-negative bacteria contain lipopolysaccharides, the major pathogenic glycolipids of the outer membrane. Glyosphingolipids, such as α-D-gluco-3-4-ceramide and α-D-galacturonosyl-3-4-ceramide of Sphingomonas, were reported to activate CD1d-restricted natural killer T (NKT) cells (34, 39, 55, 66). The action of the bacterial sphingolipids in innate immunity is attracting much attention in the field of infectious diseases.

In this context, studying the SPT proteins in these bacteria is important not only for obtaining the ideal source for crystallization of the enzyme but also for providing the structural basis for the elucidation of the biosynthetic mechanism of the unique glyosphingolipids in these bacteria. We now report the molecular cloning of three novel SPTs from sphingolipid-containing bacteria, Sphingobacterium spiritivorum EY3101^T, Sphingobacterium multivorum GTC97, and Bdellovibrio stolpii ATCC 27052. All of these bacterial enzymes were successfully overproduced in E. coli and enzymatically characterized. Their properties resembled those of the eukaryotic enzyme more closely than those of the bacterial enzymes. Thus, these SPTs, we termed GSYNYLMGF and DEAH, corresponded to the amino acid sequences GSYNYLMGF and DEAH, respectively, of the S. multivorum SPT. The PCR product was directly cloned into a pCRII vector (Invitrogen) and sequenced using a DYEnamic ET Dye Terminator cycle sequencing kit (Amersham Biosciences) and an ABI 310 DNA sequencer (Perkin-Elmer). To obtain the full-length gene, a genomic DNA library (3 × 10^7 recombinants) was screened with the 3P-labeled PCR product (500 bp) as a probe. The library was constructed as follows: genomic DNA from S. multivorum was partially digested with Sau3A1, and fragments of between 2.5 and 3.5 kb were purified by agarose gel electrophoresis and ligated into the BamHI-digested pUC18; the constructs were used to transform the E. coli JM109. Labeling of the probe and detection of the hybridizing fragments were performed using the BcaBEST labeling kit (Takara Bio, Kyoto, Japan) and Quick-Hyb hybridization solution (Stratagene), respectively. Three positive clones were isolated in the first screening, and the complete DNA sequence was determined for both strands of all three clones.

**Isolation and sequencing of genomic DNA clones encoding S. multivorum SPT.** The genomic DNA of S. multivorum was prepared according to a standard method (5). Based on the amino acid sequences of the Sphingobacterium SPT and eukaryotic LCBl/LCBr proteins, we synthesized degenerate oligonucleotides to obtain partial DNA fragments encoding the S. multivorum SPT gene by PCR with genomic DNA from S. multivorum. The oligonucleotides 5’-GG(TCAG)(TA)(CG)T(CA)(GG)(TCAG)(TA)T-3’ and 5’-CC(TCAG)(AT)(TCAG)(TG)(TA)(AG)(TG)(TCAG)(GC(TC)TC(AG)T-3’ corresponded to the amino acid sequences GSYNYLMGF and DEAH, respectively, of the Sphingobacterium SPT. PCR was performed using the LA Taq DNA polymerase (Takara Bio, Kyoto, Japan) under the following conditions: 30 cycles of 94°C for 30 s, 40°C for 30 s, and 72°C for 1 min and then 72°C for 10 min. The PCR product was directly cloned into a pCRII vector (Invitrogen) and sequenced using a DYEnamic ET Dye Terminator cycle sequencing kit (Amersham Biosciences) and an ABI 310 DNA sequencer (Perkin-Elmer). To obtain the full-length gene, a genomic DNA library (3 × 10^7 recombinants) was screened with the 3P-labeled PCR product (500 bp) as a probe. The library was constructed as follows: genomic DNA from S. multivorum was partially digested with Sau3A1, and fragments of between 2.5 and 3.5 kb were purified by agarose gel electrophoresis and ligated into the BamHI-digested pUC18; the constructs were used to transform the E. coli JM109. Labeling of the probe and detection of the hybridizing fragments were performed using the BcaBEST labeling kit (Takara Bio, Kyoto, Japan) and Quick-Hyb hybridization solution (Stratagene), respectively. Three positive clones were isolated in the first screening, and the complete DNA sequence was determined for both strands of all three clones.

**Isolation and sequencing of genomic DNA clones encoding S. spiritivorum SPT.** Genomic DNA from S. spiritivorum was prepared by ISOPLANT (Wako, Osaka, Japan) according to the manufacturer’s specifications. Based on the amino acid sequences of Sphingomonas paucimobilis and S. multivorum SPTs, we synthesized degenerate oligonucleotides to obtain partial DNA fragments encoding the S. spiritivorum SPT gene by PCR with genomic DNA from S. spiritivorum. The oligonucleotides 5’-GG(TCAG)(TA)(CG)T(CA)(GG)(TCAG)(TA)T-3’ and 5’-CC(TCAG)(AT)(TCAG)(TG)(TA)(AG)(TG)(TCAG)(GC(TC)TC(AG)T-3’ corresponded to the amino acid sequences GSYNYLMGF and DEAH, respectively, of the Sphingobacterium SPT. PCR was performed using the LA Taq DNA polymerase under the following conditions: 30 cycles of 94°C for 30 s, 40°C for 30 s, and 72°C for 1 min and then 72°C for 10 min, where T denotes that the annealing temperature was successively increased by 0.25°C for each cycle. The PCR product was cloned and sequenced. To obtain the full-length SPT gene, a genomic DNA library (3 × 10^7 recombinants) was screened with the 3P-labeled PCR product (342 bp) as a probe. The construction of the genomic DNA library, labeling of the probe, and detection of the hybridizing fragments were carried out in the same way as for the S. multivorum. Two positive clones were isolated in the first screening, and the complete DNA sequence was determined for both strands of both clones.

**Isolation and sequencing of genomic DNA clones encoding the B. stolpii SPT.** Genomic DNA from B. stolpii was prepared by using ISOPLANT according to the manufacturer’s specifications. Based on the amino acid sequences of the bacterial SPTs, we synthesized degenerate oligonucleotides to obtain partial DNA fragments encoding the B. stolpii SPT gene by PCR with genomic DNA from B. stolpii. The oligonucleotides, 5’-GG(TCAG)(TA)(CG)T(CA)(GG)(TCAG)(TA)T-3’ and 5’-CC(TCAG)(AT)(TCAG)(TG)(TA)(AG)(TG)(TCAG)(GC(TC)TC(AG)T-3’ corresponded to the amino acid sequences GSYNYLMGF and DEAH, respectively, of the S. multivorum SPT. The PCR conditions were the same as those for the S. spiritivorum SPT gene. To obtain the full-length SPT gene, a genomic DNA library (1 × 10^7 recombinants) was screened with the 3P-labeled PCR product.
Positive-ion full scan and data-dependent positive-ion tandem mass spectrometry was monitored by a ThermoElectron LCQdeca mass spectrometer equipped with in 5 min, and then to 50% solvent B and 50% solvent C in 15 min. The effluent 2-propanol, 4:6 by volume) in 6 min, to 95% solvent B and 5% solvent C sequence: from 100% solvent A to 70% solvent A and 30% solvent B (hexane: silica column (1 by 150 mm; OmniSeparo-TJ, Hyogo, JAPAN) equilibrated with solvent. Immediately after connecting the trap by valve switching to a separation the extracted lipids was applied onto the trap and then washed with the same equilibrated with solvent A (hexane containing 0.1% formic acid). An aliquot of online to the normal-phase high-performance liquid chromatography (HPLC) was applied to a DEAE-Toyopearl 650 M column (2.5 by 20 cm) equilibrated was applied onto a Butyl-Toyopearl 650 M column (2.5 by 20 cm) equilibrated with the same buffer containing 30%-saturated (NH4)2SO4. For. S. spiritivorum, SPT, the condition of 20%-saturated (NH4)2SO4 was adapted. SPT was eluted with a decreasing linear gradient of (NH4)2SO4 concentrations (30% to 0% or 20% to 0%) in 1 liter of 20 mM potassium phosphate. The pooled fractions were concentrated and then applied to a hydroxyapatite column (1.6 by 20 cm) equilibrated with 10 mM potassium phosphate (pH 7.6). The protein was eluted with a linear gradient of 10 to 250 mM potassium phosphate in 1 liter. The SPT fractions were concentrated and then applied to a Sephacryl S-200 HR column (1.6 by 80 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.6) containing 0.1 M EDTA and 150 mM NaCl. The active fractions were combined, concentrated to 2 to 5 ml, filtered, and stored at 4°C. Mass spectrometric analyses of reaction products. To identify the reaction products of the bacterial SPT, the reaction was carried out in the presence of 1.6 mg purified SPT, 20 mM L-serine, 5 mM acyl CoA, 50 mM EDTA, 50 mM HEPES, and 0.15 M KCl (pH 7.5) in a final volume of 0.1 ml. The reaction was stopped by the addition of 0.1 ml of ~2 M ammonia, and then the total lipids were extracted by the method of Bligh and Dyer (9), followed by hexane-2-propanol (3:2 [vol/vol]) and a salt solution partition (45). The lipid products were identified by electrospray ionization (ESI) ion-trap mass spectrometry connected online to the normal-phase high-performance liquid chromatography (HPLC) (30, 58). LCBs can be analyzed by a method similar to the ceramide analysis previously reported (30), with minor modifications. A trap column (1 by 20 mm) fitted to a switching valve (Valco Instruments Co., Houston, Texas) was pre-equilibrated with solvent A (hexane containing 0.1% formic acid). An aliquot of the extracted lipids was applied onto the trap and then washed with the same solvent. Immediately after connecting the trap by valve switching to a separation silica column (1 by 150 mm; Omniseparo-TJ, Hyogo, JAPAN) equilibrated with solvent A, the LCBs bound to the trap were eluted with the following gradient sequence: from 100% solvent A to 70% solvent A and 30% solvent B (hexane: 2-propanol, 4:6 by volume) in 6 min, to 95% solvent B and 5% solvent C (hexane:2-propanol:1 M ammonium formate:water, 40:60:2:24:9:76 by volume) in 5 min, and then to 50% solvent B and 50% solvent C in 15 min. The effluent was monitored by a ThermoElectron LCQdeca mass spectrometer equipped with an ESI tip, FortiTip (20-mm inside diameter and 150-mm outside diameter; Omniseparo-TJ, Hyogo, JAPAN) on an xz stage (AMR, Tokyo, Japan) in positive-ion full scan and data-dependent positive-ion tandem mass spectrometry (MS/MS) modes on a single run. An ESI voltage of 1.6 kV was used. Spectrophotometric measurements. The absorption spectra of the SPTs were recorded by a Hitachi U-3300 spectrophotometer at 25°C. The circular dichroism (CD) spectra of the SPTs were recorded by a Jasco J-720-W1 spectropolarimeter at 25°C. The buffer solution for the spectrometric measurements contained 50 mM HEPES-NaOH (pH 7.5), 150 mM KCl, and 0.1 mM EDTA. The purified enzyme was equilibrated with this buffer by gel filtration using a PD-10 (Sephadex G-25) column prior to the measurements.

Antibody preparation against each bacterial SPT. The antisera against Sphingomonas paucimobilis, S. multivorum, or B. stolpii SPT was prepared by immunization of rabbits with the purified SPT proteins. Each anti-SPT immuno- globulin G (IgG) was affinity purified with the corresponding SPT-immobilized Sepharose 4B from the total IgG fraction of the rabbit serum by a standard method. Immunoblot analysis. sodium dodecyl sulfate (SDS)-polyacrylamide gel elec- trophoresis was performed as described by Laemmli (35) with the SDS-Tris system using a 3% (wt/vol) stacking and a 10% (wt/vol) separating gel. The gels were blotted onto Immobilon-P® polyvinylidene difluoride membranes (Milli- pore, MA) using a semidyed blotting method. The membranes were blocked at room temperature for 2 h in phosphate-buffered saline (PBS) containing 1.5% (wt/vol) bovine serum albumin (BSA), followed by incubation with diluted anti- SPT rabbit antibodies at room temperature for 3 h. The membranes were washed and incubated with a 15,000 dilution of the horseradish peroxidase-conjugated goat antirabbit antibody solution at room temperature for 3 h. Visualization of the immunoreactive bands was performed using chemical luminescence (ECL detection kit; Amersham Biosciences Inc., Piscataway, NJ).

Electron microscopic analysis by the thin-section (postembedding) method. The cells were first fixed in a 0.1 M sodium cacodylate buffer (pH 7.4) containing 4% paraformaldehyde and 0.1% glutaraldehyde for 2 h at 4°C, dehydrated in a graded series of ethanol solutions (50% to 100%), and embedded in LR White resin (London Resin Co., Ltd., Hampshire, United Kingdom) overnight at 60°C. Ultrathin sections (90 nm in thickness) were prepared by an ultramicrotome (2088-V, LKB; Bromma, Sweden). The sections were transferred onto fine nickel grids and incubated in PBS containing 1% BSA and 1.5% normal goat serum for 15 min at room temperature and in PBS containing 1% BSA, 1.5% normal goat serum, and anti-SPT IgG overnight at 4°C. Subsequently, the grid was incubated in PBS containing 1% BSA, 1.5% normal goat serum, and goat anti-rabbit IgG conjugated to 10-nm gold particles for 30 min at room temperature. All sections were finally stained with uranyl acetate and lead citrate. The preparation was examined using an electron microscope, JEM2000EX (JEOL, Tokyo, Japan).

Other methods. SPT activity was measured according to previously described methods (27). The protein concentration during the purification procedure was determined using a BCA protein assay kit (Pierce Chemical) with bovine serum albumin as the standard. The protein concentration between the purification procedure was spectrophotometrically determined using the following molar extinction coefficients at 280 nm for the PLP form of each enzyme: 2.83 cm−1 M−1 for S. paucimobilis SPT, 2.68 cm−1 M−1 for S. spiritivorum SPT, and 1.98 cm−1 M−1 for B. stolpii SPT.

Nucleotide sequence accession numbers. The nucleotide and protein sequences of the spt genes have been submitted to the GenBank database (S. multivorum, AB259214; S. spiritivorum, AB259215; B. stolpii, AB259216).

RESULTS

SPT activity in S. multivorum and B. stolpii. We previously reported that both Sphingomonas paucimobilis SPT and S. spiritivorum SPT are water-soluble enzymes (27). The SPT activities in S. multivorum and B. stolpii were examined in a similar way (27), and the results are presented in Fig. 1 together with those for Sphingomonas paucimobilis, S. spiritivo- rum, and the mouse liver microsome. For all of the bacterial strains, SPT activity was found in both the supernatant and precipitate fractions, which were prepared by ultracentrifugation. The distribution profile of the activity in the supernatant and the precipitate seems to vary depending on the species. However, since the precipitate fraction contains a nonnegli- gible amount of unlysed cells, it is hard to precisely estimate how much of the SPT activity is associated with the membrane. This issue was morphologically assessed using immunoelectron microscopy, as described later in detail.

Cloning of SPT genes from S. multivorum, S. spiritivorum, and B. stolpii. The three SPT genes (spt) from S. multivorum, S. spiritivorum, and B. stolpii were cloned by degenerate PCR and genome library screening, as described in Materials and Meth-
FIG. 1. Thin-layer chromatography of radiolabeled products obtained by SPT assay reactions of mouse liver microsomes, S. paucimobilis, S. multivorans, S. spiritivorum, and B. stolpii. The assay reactions and thin-layer chromatography were carried out as described in reference 27. Reaction products formed in the presence of the crude extract, the precipitate (resuspended), or the supernatant were spotted. The volume of the crude extract used for the reaction was 100 μl (140 mg protein/ml), and the amounts of the precipitate and the supernatant were those obtained from the same volume (100 μl) of the crude extract. Lanes 1 and 8, mouse liver microsomes as a reference; lanes 2, 5, 9, and 12, crude extracts after sonication of S. paucimobilis (lane 2), S. multivorans (lane 5), S. spiritivorum (lane 9), and B. stolpii (lane 12); lanes 3, 6, 10, and 13, the precipitates after centrifugation at 1,000,000 × g of the crude extracts of S. paucimobilis (lane 3), S. multivorans (lane 6), S. spiritivorum (lane 10), and B. stolpii (lane 13); lanes 4, 7, 11, and 14, the supernatants after centrifugation at 100,000 × g of the crude extracts of S. paucimobilis (lane 4), S. multivorans (lane 7), S. spiritivorum (lane 11), and B. stolpii (lane 14).

odds. Properties of the gene products of the bacterial SPTs are summarized in Table 1.

Sequence comparisons. The amino acid sequence alignment of the human SPT subunits, SPLTC1/SPLTC2/SPLTC3, products of isolated bacterial SPT genes and the putative SPT gene of Zymomonas mobilis ZM4 (50), and two other enzymes of the α-oxamine synthase family, i.e., E. coli 8-amino-7-oxononanate synthase (AONS) and human 5-aminolevulinate synthase (ALAS), is shown in Fig. 2A. AONS and ALAS were selected as the proteins most and second most homologous to SPT, respectively. An overall sequence similarity was found between these proteins. Bacterial SPTs are highly similar to each other, and conserved amino acids are distributed throughout the entire sequences of the polypeptides. The S. multivorans SPT shared the highest amino acid sequence identity (87.7%) with the S. spiritivorum SPT. The Z. mobilis SPT also showed a high identity (73.1%) with the Sphingomonas SPT. The amino acid sequence identities among other bacterial SPTs were 35.0 to 48.2%. The conservation of the amino acid sequences between each bacterial enzyme was higher than that between the bacterial SPTs and human SPLTC proteins. S. multivorans SPT is 25%, 31%, and 32% identical, S. spiritivorum SPT is 27%, 32%, and 33% identical, and B. stolpii SPT is 24%, 33%, and 34% identical to human SPLTC1, SPLTC2, and SPLTC3, respectively. Several small hydrophobic stretches of amino acids were distributed throughout the bacterial SPT protein; however, no obvious transmembrane region(s) were found. The SPT-specific PLP-binding motif (GTFKSXXXG) is completely conserved among all the bacterial SPTs.

A phylogenetic tree of the SPTs was constructed by the neighbor-joining method using the E. coli AONS protein as an outgroup (Fig. 2B). The selection of this protein as the outgroup was done because E. coli AONS is the protein apparently distinct from SPT but has the highest similarity to SPT among the α-oxamine synthase family enzymes. The bootstrap values at the nodes, except for the leftmost two nodes, were all 100%. The reason for the relatively low values of 74% and 82% of the two nodes is not clear, but it may be partially attributed to the use of the evolutionarily distant (i.e., functionally different) outgroup. The divergence of the bacterial SPTs reflects the phylogeny of the bacteria; Sphingomonas paucimobilis and Z. mobilis belong to the same family of bacteria, called Sphingomonadaceae, and S. multivorans and S. spiritivorum belong to the family Sphingobacteriaceae (50, 69). The branch lengths of the LCB1 proteins are significantly greater than those of other proteins, including the LCB2, SPTLC3, and bacterial SPTs, suggesting relatively higher evolution rates of the LCB1 proteins. S. spiritivorum SPT is the nearest relative to the mammalian LCB2 proteins, followed by the SPTs from S. multivorans, B. stolpii, Z. mobilis, and Sphingomonas paucimobilis.

Overproduction and purification of recombinant SPTs. Each bacterial SPT has been stably overexpressed as a soluble protein in E. coli. The expression levels of the recombinant proteins reached approximately 10 to 20% of the total protein of E. coli cells without growth inhibition of the expression host. While the B. stolpii SPT was most abundantly expressed, half of the expressed protein formed inclusion bodies. In order to increase the solubility of the recombinant enzyme, we made a deletion variant of the B. stolpii SPT lacking the N-terminal 13 amino acid residues. The addition of 20% (wt/vol) glycerol was necessary to prevent the precipitation of the B. stolpii SPT during purification and storage. All of the recombinant enzymes were purified to homogeneity by column chromatography in three steps, and the purified SPTs showed a single protein band with an apparent Mr of approximately 45,000 for the S. multivorans and S. spiritivorum SPTs and 50,000 for the B. stolpii SPT, respectively, by SDS-polyacrylamide gel electrophoresis (data not shown). About 20 mg of purified enzyme was routinely obtained from 1-liter cultures in each case and could be stored at 4°C for more than 6 months.

Physicochemical characterization. The Mr values of all three bacterial SPTs were estimated to be 90,000 by gel filtration. Matrix-assisted laser desorption ionization–time-of-flight MS analyses gave a signal at m/z 43,645 for the S. multivorans SPT, 43,780 for the S. spiritivorum SPT, and 44,397 for the B. stolpii SPT lacking the N-terminal 13 amino acid residues. These values were in good agreement with the values of 43,640, 43,797 and 44,522, which were calculated from the deduced amino acid sequences of each recombinant enzyme without the first methionine within experimental error. These results show

TABLE 1. Bacterial SPTs

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>GenBank accession no. of SPT</th>
<th>ORF size (bp)a</th>
<th>[G + C] (%)</th>
<th>Mr</th>
<th>pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. paucimobilis</td>
<td>AB055142</td>
<td>1,263</td>
<td>65.00</td>
<td>45,041</td>
<td>5.66</td>
</tr>
<tr>
<td>S. multivorans</td>
<td>AB259214</td>
<td>1,200</td>
<td>38.33</td>
<td>43,771</td>
<td>5.05</td>
</tr>
<tr>
<td>S. spiritivorum</td>
<td>AB259215</td>
<td>1,200</td>
<td>41.08</td>
<td>43,929</td>
<td>4.97</td>
</tr>
<tr>
<td>B. stolpii</td>
<td>AB259216</td>
<td>1,263</td>
<td>43.51</td>
<td>46,172</td>
<td>6.25</td>
</tr>
</tbody>
</table>

a ORF, open reading frame.
that these bacterial SPTs have dimeric structures composed of two identical subunits.

The pH stability of the purified SPTs was investigated (data not shown). This information is necessary for both crystallization and storage. Enzymes from both S. spiritivorum and B. stolpii SPTs showed an above-90% activity in the pH range of 6.8 to 8.5, with an optimum pH at 7.0 to 8.0. Only the S. multivorum SPT was denatured below pH 7.2, and its optimum pH was 7.4 to 8.0.

FIG. 2. Sequence alignment and molecular phylogenetic tree of SPTs. (A) Aligned sequences of bacterial SPTs, human SPT subunits (SPTLC1, SPTLC2, and SPTLC3), E. coli AONS, and human ALAS2. The deduced amino acid sequences of the SPTs and other proteins were aligned using the CLUSTALX version 1.83 program (59). The gap opening and extension parameters were set to 10 and 0.2, respectively. Zymomonas SPT, Zymomonas mobilis SPT; Sphingomonas SPT, Sphingomonas paucimobilis SPT; S. multivorum SPT, Sphingobacterium multivorum SPT; S. spiritivorum SPT, Sphingobacterium spiritivorum SPT; Bdellovibrio SPT, Bdellovibrio stolpii SPT. Residues identical among all the proteins are in light blue, and those conservatively substituted are in green. Residues indicated by the reverse triangle are active-site residues that are considered to be important for catalysis. The red-boxed sequences are the SPT-specific PLP-binding motif (GTFSKSXXXXGG). (B) Molecular phylogenetic tree of SPTs from various sources. The phylogenetic tree was constructed by the neighbor-joining method using the E. coli AONS protein as an outgroup. The number at each node represents the bootstrap value as a percentage of 1,000 replications.
All purified recombinant SPTs showed characteristic absorption spectra of PLP-dependent enzymes (Fig. 3). For all of the bacterial SPTs, the intensities of the absorption peaks were not changed by the pH. The shapes of the spectra of the two Sphingobacterium SPTs were different from that of the Sphingomonas enzyme (Fig. 3A, B, and C). They had only a single peak at 426 nm in addition to the protein absorption peak at 278 nm. On the other hand, the B. stolpii SPT showed two peaks, at 338 and 426 nm (Fig. 3D), and in this respect was similar to the Sphingomonas enzyme, although the relative intensities of the two peaks are different. These absorption peaks, respectively, correspond to the enolimine and ketoenamine forms of the internal Schiff base (aldimine formed between the aldehyde group of PLP and the ε-amino group of a lysine residue in the active site) of SPT. The addition of L-serine to the purified SPTs gave rise to an intense absorption band at 426 nm for all of the SPTs and a weak band at 338 nm for the Sphingomonas and B. stolpii SPTs. These spectral changes showed hyperbolic dependencies on the concentrations of L-serine, and the apparent dissociation constants (\(K_D\)) for L-serine were calculated to be 0.47, 1.20, and 2.55 mM, respectively, for the S. multivorum, S. spiritivorum, and B. stolpii SPTs (Table 2). The CD spectra of these bacterial SPTs showed positive bands at 426 nm (and additionally at 338 nm for B. stolpii SPT), corresponding to the absorption spectra of each enzyme (data not shown). The CD spectra in the presence of a saturating amount of L-serine showed a negative band at 426 nm (data not shown). These results indicate that the added L-serine formed the external Schiff base (aldimine formed between PLP and extraneously added amino acid) with PLP. The addition of the second substrate, palmitoyl CoA, to the B. stolpii SPT, which is saturated with L-serine, resulted in a slight decrease in the 426-nm peak and the appearance of an absorption band at 515 nm (Fig. 3C). The formation of the 515-nm peak was transient, and the peak vanished within a few minutes. Such spectral changes were not observed for the Sphingobacterium SPTs or the Sphingomonas SPT.

**Identification of reaction products of bacterial SPTs.** The formation of KDS by bacterial enzymes was confirmed by HPLC/ESI–ion-trap mass spectrometry (Fig. 4). Figure 4A shows the ion chromatograms (m/z 300) of the SPT reaction product in the positive-ion mode. The most abundant ions at m/z 300 (Fig. 4C) corresponded to the protonated molecular ions [M + H]⁺ of C18:0 KDS formed from L-serine and palmitoyl CoA, the structure of which is shown in Fig. 4B. This LCB

**TABLE 2. Kinetic parameters of bacterial SPTs**

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>(K_D) (Ser) (mM)</th>
<th>(K_m) (Ser) (mM)</th>
<th>(K_m) (palmitoyl CoA) (mM)</th>
<th>(k_{cat}) (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. paucimobilis</td>
<td>1.40 ± 0.10</td>
<td>4.7 ± 0.6</td>
<td>0.69 ± 0.09</td>
<td>2.3 ± 0.11</td>
</tr>
<tr>
<td>S. multivorum</td>
<td>0.47 ± 0.10</td>
<td>4.8 ± 0.6</td>
<td>0.10 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>S. spiritivorum</td>
<td>1.20 ± 0.03</td>
<td>5.0 ± 0.8</td>
<td>0.39 ± 0.04</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>B. stolpii</td>
<td>2.55 ± 0.12</td>
<td>3.7 ± 0.4</td>
<td>ND(*)</td>
<td>0.03 ± 0.002°</td>
</tr>
</tbody>
</table>

* ND, not determined.
* ° v[\(E_i]\] value in the presence of 100 µM of palmitoyl CoA.
structure was confirmed by on-line MS/MS. A positive-ion MS/MS spectrum of the \( m/z \)-300 ion showed the presence of ions of \( m/z \) 282 and 270, arising from the neutral loss of H\(_2\)O and HCHO, respectively (Fig. 4B and D).

**Catalytic properties of bacterial SPTs.** Like eukaryotic enzymes, all of the bacterial SPTs used only the L configuration of serine as an amino acid substrate. The bacterial enzymes exhibited a broad substrate specificity concerning the chain length and the degree of unsaturation of acyl CoA (Fig. 5). Figure 5A shows ion chromatograms of the reaction product formed by the *S. multivorum* SPT. In each chromatogram, a single ion peak of the reaction product with a molecular weight corresponding to the value expected from the chain length of the acyl CoAs was detected. The longer chain length of KDS gave a slightly shorter retention time. Figure 5B shows the MS data in which the molecular ion at \( m/z \) 356 corresponded to \([M+H]^+\) of C\(_{22}\)KDS formed from L-serine and arachidoyl CoA. As shown in Fig. 5C, the MS/MS spectrum of the \( m/z \)-356 ion indicated the presence of ions of \( m/z \) 338, 326, and 320, arising from the neutral loss of H\(_2\)O, HCHO, and 2H\(_2\)O, respectively. The structures of the other KDS with different chain lengths were also confirmed in the same way.

Figure 6 shows the substrate concentration dependency of the reaction rate of the bacterial SPTs. It has been reported that eukaryotic SPT is inhibited by palmitoyl CoA concentrations greater than 50 \( \mu \)M (20, 53). A similar phenomenon was observed with the *B. stolpii* SPT, which was significantly inhibited by palmitoyl CoA concentrations greater than 100 \( \mu \)M (Fig. 6C). The SPTs of *S. multivorum* and *S. spiritivorum* were not inhibited by the high concentrations of palmitoyl CoA. For the last two enzymes, we could analyze the experimental data under steady-state conditions according to the ordered Bi-Bi mechanism (Fig. 6A and B) (49), and the kinetic parameters were obtained (Table 2). For all the enzymes, the \( K_m \) values for L-serine were in the range of 3 to 5 mM. The \( k_{cat} \) values of the *S. multivorum* and *S. spiritivorum* enzymes are apparently lower than that of the *S. paucimobilis* enzyme. The \( K_m \) values for palmitoyl CoA were 0.1 to 0.7 mM, except for the *B. stolpii* enzyme. For the *B. stolpii* enzyme, the \( K_m \) value for palmitoyl CoA and \( k_{cat} \) value could not be obtained due to the substrate inhibition by palmitoyl CoA. For comparison with other data, the \( v/[E_0] \) value in the presence of 100 \( \mu \)M palmitoyl CoA, where the maximum activity is obtained, is shown.

**FIG. 4.** HPLC/ESI-ion-trap mass spectroscopy of reaction products of bacterial SPTs. (A) Ion chromatography of the reaction products of bacterial SPTs. (B) The structure of KDS, position of fragmentation, and the size of the fragment ion are indicated. (C) MS data for the reaction product of the *S. multivorum* SPT. (D) MS-MS data for the reaction product of the *S. multivorum* SPT.
Intracellular localization of bacterial SPTs (immunolocalization). In order to examine the localization of the SPTs in the intact cell, specific polyclonal antibodies were prepared using the recombinant enzymes. As shown in Fig. 7, each antibody specifically recognized its corresponding SPT. There was no signal in the lanes containing the E. coli lysate transformed with the empty vector (Fig. 7, lanes 1, 4, and 7). The anti-S. multivorum SPT antibody cross-reacted with the S. spiritivorum SPT (data not shown). Considering the phylogenetic similarity of S. spiritivorum to S. multivorum, which might yield similar morphological results, and the technical constraints on the morphological analysis of this weak pathogenic species, S. spiritivorum was excluded from further analyses. For the B. stolpii lysate, the antibody recognized two bands, of approximately 50 kDa and 48 kDa, the latter being the same size as the recombinant SPT lacking the N-terminal 13 amino acid residues (Fig. 7, lanes 8 and 9). The thin sectioned profiles of the whole bacterial cells of Sphingomonas paucimobilis, S. multivorum, and B. stolpii are presented in Fig. 8A, B, and C, respectively. Sphingomonas paucimobilis cells have a rod shape, and the average size (width) of the cells was 0.8 μm (Fig. 8A). The cytoplasm of the Sphingomonas paucimobilis cells was characterized by the presence of clearly identifiable ribosome particles and a nonnucleoid electron-dense area. The cell envelope consisted of a one-electron-dense bilayer as an outer membrane and one additional bilayer structure as an inner membrane. The ribosome particles showed a condensed distribution near the inner membrane or the nonnucleoid electron-dense area. The shape of the S. multivorum cells was also rod type, and the average size (width) was 0.45 μm (Fig. 8B). The S. multivorum cells were slightly shorter than the Sphingomonas paucimobilis cells and had no flagella. At the inside of the cell wall, a multilayered inner membrane structure was observed. The B. stolpii cells had the shape of curved rods or spheres with a rugged cell wall (Fig. 8C). The average size (width) of the cells was 0.36 μm. As with Sphingomonas paucimobilis and S. multivorum, a multilayered cell membrane structure was seen inside of the cell wall of the B. stolpii cells. The ribosome particles were widely distributed in the electron-dense cytoplasm. In the middle of the cytoplasm, an organelle-like multilayered membrane structure was observed. The intracellular localization of SPT was analyzed for each bacterium using immunoelectron microscopy. The immunogold-labeled SPT was readily detectable as a spot-like distribution throughout the cytoplasm in the Sphingomonas paucimobilis cells (Fig. 8D). The nonnucleoid electron-dense area observed in these bacterial cells was more intensely immunostained. Some of the immunogold clusters seemed to localize near the inner membrane of the cell. Sparse immunogold particles were also detected on the outside of the cell. In the S. multivorum cells, about 88% of the immunogold-labeled SPT was distributed near the inner membrane of the cell, and the remaining imm-
munogold particles were detected in the center of the cell (cytoplasm) (Fig. 8E). In the *B. stolpii* cells, the immunogold-labeled SPT was detected in a spot-like pattern predominantly concentrated in a limited region near the inner membrane or organelle-like multilayered membrane structure (Fig. 8F). When each primary antibody had been preabsorbed with the corresponding SPT protein, the signals disappeared or at least became very faint (Fig. 8G to I).

**DISCUSSION**

Our recent efforts to crystallize SPT using the recombinant enzyme from *Sphingomonas paucimobilis*, which we had previously characterized, showed that the stability of the *Sphingomonas* SPT was not sufficient for crystallization and structural analysis. To obtain a suitable protein for crystallization, we searched for SPTs from other bacterial sources. *S. multivorum*, *S. spiritivorum*, and *B. stolpii* have been reported to contain large amounts of sphingolipids as cell membrane components (47, 62). Their SPT activities in the cytosolic fractions were comparable to that of *Sphingomonas paucimobilis*. Based on the amino acid sequences of the conserved regions between *Sphingomonas* SPT and the eukaryotic LCB1/LCB2 proteins, we carried out degenerate PCR and genomic library screening. Three novel SPT genes were isolated from these bacteria. Each recombinant protein catalyzed the KDS formation from L-serine and palmitoyl CoA (Fig. 4), confirming that the products of all of the cloned genes have SPT activity.

Catalytically important amino acid residues are conserved in bacterial SPTs. SPT belongs to the P4H/9251-oxamine synthase family of the PLP-dependent enzymes, which includes ALAS, 2-amino-3-ketobutyrate ligase (KBL), and AONS (1, 2, 4, 32, 48, 63). Bacterial SPTs show about a 30% identity with other members of this family. Previous X-ray crystallography on AONS and KBL from *E. coli* suggested catalytically important active-site residues that interact with PLP and are completely conserved in all of the bacterial SPTs. These conserved residues include (in *S. multivorum* numbering) Lys244, which forms a Schiff base linkage with PLP, Asp210, which forms a salt bridge/hydrogen bond with the pyridine N of PLP, His213, which stacks with the pyridine ring of PLP (Fig. 2A). The structures of the complexes of AONS and KBL with substrate analogues suggested that Asn52 and Arg367 are the potential hydrogen-bonding partners of the carboxylate group of the substrate L-serine in the external aldimine complex of SPT. Arg367 is indeed conserved in all of the cloned genes have SPT activity.
The sequence similarities between the bacterial SPTs and human LCB1 are higher than those between the bacterial enzymes and human LCB1. The active-site residues described above are not conserved in the bacterial enzymes, including LCB2. Apparently, LCB1 does not have a catalytic function. This is consistent with the longer branch length of LCB1, because the lack of functional constraints on the protein is considered to accelerate the evolution rate.

**Human hereditary sensory neuropathy type I-related mutation site of bacterial SPT.** The single mutations (C133Y, C133W, V144D, or G387A) in human LCB1 cause HSN1, which is the most common hereditary disorder of peripheral sensory neurons (6, 11, 61). It remains elusive how the LCB1 mutations cause changes in the SPT activity of the heterodimer and how these changes participate in the neurodegenerative symptoms in HSN1. The dominant-negative inhibition of the SPT activities by overexpression of the HSN1-related LCB1 mutants in yeast, CHO cells, and transgenic mouse strongly supports the idea that the neurodegeneration in HSN1 is related to a decrease in the sphingolipid synthesis (7, 17, 40). However, there is a claim that the remaining SPT activity is sufficient for the normal sphingolipid metabolism and viability of the HSN1 patient cells (12). An alternative mechanism is that the HSN1 mutations of LCB1 accelerate the aggregation of the SPT protein induced by hypoxia in the human lymphocytes, which leads to nonapoptotic death (13). Contrary to these observations, de novo glucosyl ceramide synthesis increased in the lymphoblast cell lines from HSN1 patients (11), suggesting that the neural degeneration in HSN1 is due to the overproduced ceramide or the abnormal cellular lipid composition. Cys133 and Val144 of human LCB1 correspond to Cys78 and Ile90, respectively, of *S. multivorum* SPT. These residues are also conserved in the SPTs of *S. spiritivorum* and *B. stolpii* but not in *Sphingomonas paucimobilis* and *Z. mobilis* sequences, in which Cys133 and Val144 correspond to Thr and Asp/Gly, respectively. It should be noted that *Sphingomonas* SPT, which carries an amino acid substitution equivalent to the HSN1-type mutation (Asp at the position of Val144 in human Lcb1p), has a much higher activity than other bacterial enzymes carrying a hydrophobic amino acid (Ile) at that position. These findings lead us to speculate on the possibility that the...
HSN1-type mutations in human LCB1 may result in some toxic gain of function, such as increased activity toward normal or abnormal acyl CoAs.

Immunolocalization of bacterial SPT as a peripheral membrane protein. All of the bacterial SPTs examined so far are water-soluble homodimeric enzymes. Their water-soluble character is the most different aspect in relation to the membrane-bound enzymes of the eukaryotes. The reaction product of SPT, KDS, is a very hydrophilic sphingolipid intermediate that is easily incorporated into membranes. While membrane localization of the eukaryotic SPT complex seems very reasonable, the characteristics of the bacterial SPT as a water-soluble protein in vitro raised questions about the mechanism of the product release or the interaction with the cellular membrane in vivo. Therefore, we further analyzed the intracellular localization of the bacterial SPT by immunoelectron microscopy (Fig. 8). These results demonstrated the limited distribution of SPT molecules in bacterial cells in a spot-like pattern (Sphingomonas paucimobilis) or a clearly condensed pattern near the inner membrane of the cells (S. multivorans and B. stolpii). These results are different from the homogenous distribution pattern of the general soluble proteins. S. multivorans and B. stolpii SPT may loosely bind to the inner membrane of bacterial cells like a peripheral membrane protein or may indirectly interact with the membrane via some anchor protein in vivo. The KDS released from these SPTs may directly enter the inner membrane and then be efficiently converted into various glycosphingolipids as the final products by other modification enzymes, which may be present in the bacterial cell membrane.

Most eukaryotic-like SPT from B. stolpii. The SPT from B. stolpii is different from the other bacterial SPTs in the following ways. First, during purification, the recombinant enzyme required the addition of 20% glycerol, which is the most general stabilizer for membrane proteins. Second, B. stolpii has an organelle-like multilayered membrane structure within the cell, and the immunoreaction to the native SPT was detected on the cytosolic face of the organelle-like structure. Third, the reaction rate is the lowest among the bacterial SPTs examined and is on the same order as the eukaryotic enzymes. Fourth, when both L-serine and palmitoyl CoA were added to the enzyme, a transient accumulation of the quinonoid intermediate was spectroscopically detected, suggesting a slow catalytic turnover of the enzyme. Finally, inhibition by palmitoyl CoA as seen in the mammalian SPTs was observed. These data indicate that the B. stolpii SPT is the most eukaryotic-like enzyme among the bacterial SPTs, although it is a bacterial homodimeric SPT and in this respect different from the heterodimeric enzymes of the eukaryotes.

The physiological function of the sphingolipids in bacteria is unknown except for their role as a main component of the bacterial cell membrane. The broad acyl CoA specificity of bacterial SPT might be advantageous to bacteria in that they can escape from the influence of the environmental changes on bacterial envelope properties that affect their survival. If the fatty acids available to the bacterial cells are changed, their SPTs can utilize other acyl CoAs with different chain lengths for LCB synthesis. On the other hand, there is no current report that these organisms produce sphingolipids of various LCB lengths depending on the substrate fatty acids to which they are exposed. Considering that the 14C-labeled palmitic acid in the culture medium is selectively incorporated into the LCB of Sphingomonas under normal conditions (70), it seems more probable that these organisms make essentially the same sphingolipid LCBs regardless of the fatty acid composition in the environment. This is a future research topic.

There is an interesting report demonstrating that the predatory bacterium Bdellovibrio bacteriovorus UKi2 contains phosphophingolipids, and the mutant strain UKi1, lacking sphingolipids, loses its parasitic ability (56). The sphingolipids of Bdellovibrio may be associated in some way with the ability of this bacterium to attack and grow on suitable bacterial hosts. It has been reported that the glycosphingolipids from Sphingomonas sp. and Ehrlichia muris were recognized by the CD1d-restricted NKT cells in the mouse and human, which provide an innate-type immune response (34, 39, 55, 66). We have found putative SPT genes among the genome databases of some pathogenic bacteria and have already examined the SPT activities of these gene products. These pathogenic bacteria may also have cell walls containing glycosphingolipids that serve as direct targets for the NKT cells. Another example of the possible involvement of sphingolipids in pathogenesis is shown by a recent finding that the marine planktonic pathogen Coccolithovirus EhV-86 has a cluster of genes highly homologous to the enzymes of the sphingolipid metabolism (65) and these genes are coordinately expressed within 2 h of infection (3). This viral SPT is a unique monomeric enzyme, which is composed of two separable domains, suggesting a fused heterodimer corresponding to the eukaryotic SPTs (18). This viral SPT expressed in the yeast cell was localized to the endoplasmic reticulum and preferred myristoyl CoA (C14) to palmitoyl CoA (C16) as the substrate. It was suggested that the viral SPT may alter the sphingolipid metabolism of the host during pathogen infection.

We obtained novel SPT molecules with various characteristics, from the highly stable and water-soluble type to the eukaryotic-like and loosely membrane-bound type. Recently the recombinant SPT from S. multivorans yielded crystals of sufficiently good quality for X-ray crystallographic analysis. Further structural analyses of the SPT complex with the substrate, product, or analogues are now under way. Not only the S. multivorans SPT but also other bacterial enzymes will be useful because they have the characteristic spectroscopic features reflecting the intermediate accumulation of each step in the catalytic cycle. The reaction mechanism of SPT could be clarified in the context of the three-dimensional structure of the bacterial SPT. Information obtained from the bacterial enzymes will provide clues to the reaction mechanism of the more complex eukaryotic homologue.

ACKNOWLEDGMENTS

We thank Eiko Yabuchi of Aichi Medical University, Yoshiaki Kawamura of Aichi Gakuin University, and Yoko Watanabe of Niigata University for generously providing bacterial strains. We also acknowledge Nobuyoshi Esaki and Tatsuo Kurihara of Kyoto University for useful comments on the immunoelectronmicroscopic study.

This work was supported by a Grant-in-Aid for Encouragement of Young Scientists (B), 16770103, and a Grant-in-Aid for Scientific Research (C), 18570114 (to H.I.), from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by a Grant-in-Aid for Scientific Research (C) 16570125 (to H.H.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.


