Serine palmitoyltransferase (SPT, EC 2.3.1.50) is a key enzyme in sphingolipid biosynthesis and catalyzes the decarboxylative condensation of L-serine and palmitoyl-coenzyme A to 3-ketodihydrosphingosine. We found that the Gram-negative obligate aerobic bacteria *Sphingomonas paucimobilis* EY2395 have significant SPT activity and purified SPT to homogeneity. This enzyme is a water-soluble homodimeric protein unlike eukaryotic enzymes, known as heterodimers composed of tightly membrane-bound subunits, named LCB1 and LCB2. The purified SPT shows an absorption spectrum characteristic of a pyridoxal 5'-phosphate-dependent enzyme. The substrate specificity of the *Sphingomonas* SPT is less strict than the SPT complex from Chinese hamster ovary cells. We isolated the SPT gene encoding 420 amino acid residues (M, 45,041) and succeeded in overproducing the SPT protein in *Escherichia coli*, in which the product amounted to about 10–20% of the total protein of the cell extract. *Sphingomonas* SPT shows about 30% homology with the enzymes of the α-oxamine synthase family, and amino acid residues supposed to be involved in catalysis are conserved. The recombinant SPT was catalytically and spectrophotometrically indistinguishable from the native enzyme. This is the first successful overproduction of an active enzyme in the sphingolipid biosynthetic pathway. *Sphingomonas* SPT is a prototype of the eukaryotic enzyme and would be a useful model to elucidate the reaction mechanism of SPT.

Sphingolipids are ubiquitous membrane components having a backbone structure called long-chain base (LCB) which is N-fatty acylated and linked to various polar head groups. In eukaryotes, sphingolipids such as sphingosine, sphingosine 1-phosphate, and ceramide are known to play important roles as second messengers in various cellular events including proliferation, differentiation, senescence, apoptosis, and immune response (1).

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 sphingolipid biosynthesis, utilizing substrates that are shared by other metabolic pathways, and has an activity lower than those of other enzymes involved in sphingolipid biosynthesis. Therefore, SPT is thought to be a rate-determining enzyme in the sphingolipid synthetic pathway and, accordingly, a key enzyme regulating the cellular sphingolipid content (2). Eukaryotic SPTs have been known as membrane-bound proteins, enriched in the endoplasmic reticulum with their catalytic sites facing the cytosol (3). Genetic studies have shown that two different genes, *LCB1* and *LCB2*, are required for SPT activity in the yeast *Saccharomyces cerevisiae* (4–6). Subsequently, mammalian homologues of the *LCB* genes from mouse, human, and Chinese hamster ovary cells have also been reported (7–9). The biochemical studies using the Chinese hamster ovary cell mutants demonstrated that both the *LCB1* and *LCB2* proteins are subunits of SPT (10, 11). There is a high sequence homology between *LCB1* and *LCB2*, and they are classified as new members of the PLP-dependent α-oxamine synthase subfamily (6). Based on the finding that *LCB1* does not have a PLP-binding motif while *LCB2* carries a lysine residue expected to form a Schiff base with PLP, *LCB1* and *LCB2* have been speculated as a regulatory unit and a catalytic unit, respectively (6, 10, 11). There is, however, no biochemical explanation for the regulation mechanism of the SPT reaction at present. The purification of the native form SPT from any eukaryotes has not been successful because of the extremely low content and instability of this enzyme. We, too, tried to construct the expression system of the mouse SPT complex in *Escherichia coli* (12). Affinity tagged forms of mouse LCB proteins lacking the membrane-anchor regions were coexpressed in *E. coli* as partially soluble proteins, but the purified SPT complex did not show enzymatic activity. As the only achievement, Hanada et al. (11) obtained an active SPT complex from the Chinese hamster ovary cell mutants expressing a hexahistidine-tagged *LCB1* protein. However, it is very difficult to obtain a sufficient amount of the active enzyme for detailed enzymological analysis from such purification sources.

Although sphingolipids are not typical membrane constituents in prokaryotes, there are some exceptions. In strict anaerobes such as the genera *Bacteroides*, *Porphyromonas*, and *Prevotella*, high levels of sphingolipids were found; in some species their contents in the total extractable lipid came to 70% (13, 14). It has been reported that *Bacteroides melaninogenicus* contains a water-soluble SPT, but the purification of this enzyme was not successful (15). The Gram-negative obligatory
aerobic bacteria *Sphingomonas* and *Sphingobacterium* are the genera whose lipid composition and structure of their sphingolipids have been investigated most extensively (16, 17). In cells of *Sphingomonas paucimobilis*, the lipopolysaccharide in their outer membrane is completely substituted by sphingoglycolipid, and its proposed structure is 1-O-(3-glucuronosyl-2-N-2'-hydroxytryosylidihydrophosphogine) (glucuronosyl ceramide) (18, 19).

We found that cells of *S. paucimobilis* YE2395\(^T\) and *Sphingobacterium spiritivorum* YE3101\(^T\) contain significant SPT activity and report here the purification to homogeneity and characterization of SPT from *S. paucimobilis* YE2395\(^T\).

### EXPERIMENTAL PROCEDURES

#### Chemicals—L-Serine and other natural l-amino acids were obtained from Nacalai Tesque (Kyoto, Japan). Palmitoyl-CoA and lauroyl-CoA were from Funakoshi (Tokyo, Japan). Myristoyl-CoA, n-heptadecanoyl-CoA, palmitoyl-CoA, oleoyl-CoA, O-phospho-l-serine, o-methyl-l-serine, and l-serine methylster were from Sigma. Serinol and serinamide were from Aldrich. 3-Hydroxypropanic acid was from Tokyo Kasei Kogyo (Tokyo, Japan). 4-(2-Aminoethyl)-benzenesulfonyl fluoride (AEBSF) was from Roche Molecular Biochemicals. The low molecular weight gel filtration calibration kit, gel filtration calibration kit, phenyl- Sepharose CL-4B, PD-10, Superose\(^TM\) 12, and MonoQ\(^TM\) HR 5/5 were from Amersham Pharmacia Biotech. DEAE-Toyopearl 650M and Butyl-Toyopearl 650M were from Tosoh (Tokyo, Japan). The CHT-II Econo-Pac\(^TM\) cartridge was from Bio-Rad Laboratories. Competent *E. coli* JM109 was purchased from Nippon Gene (Tokyo, Japan). *E. coli* BL21(DE3) pLysS and plasmid pET21b were from Novagen. A plasmid pUC118 was from TaKaRa (Kyoto, Japan). All other chemicals were of the highest grade commercially available.

#### Bacterial Strains and Growth Conditions—*S. paucimobilis* YE2395\(^T\) and *S. spiritivorum* YE3101\(^T\) were gifts from Dr. Eiko Yabuuchi, Aichi Medical University, Aichi, Japan. Each strain was grown in 1 liter of LB medium in 5-liter flasks at 30 °C and 90 rpm. Cells were harvested in the late exponential growth phase (after 8–9 h) and stored at −20 °C before use.

#### Assay of the Enzyme Activity—In the purification steps, the SPT activity was measured according to the methods of Williams et al. (20) with minor modifications. The enzyme solution was incubated in 100 µl of a standard SPT reaction buffer (100 mM HEPES-NaOH buffer (pH 7.5) containing 0.1 mM EDTA, 5 mM dithiothreitol, 0.1 mM AEBSF, and 20 μl PLP. The dialysate was applied to a CHT-II column (3.5 cm), which had been connected to a fast protein liquid chromatography system, equilibrated with the buffer solution for the absorption measurements contained 50 mM Tris-HCl buffer (pH 7.5) containing 150 mM NaCl and eluted with a 25-ml linear gradient from 150 to 200 mM NaCl (pH 7.5). The active fractions were combined, concentrated to 1 ml, filtered, and stored at 4 °C. When 30 g of cells were used as the starting material, 350 μg of a pure preparation of SPT was obtained.

#### Amino Acid Sequencing—The purified enzyme (8.9 nmol) was carboxymethylated, desalted, and digested at 37 °C for 30 min with lysyl endopeptidase (33 pmol). The digested peptides were isolated by reversed-phase high performance liquid chromatography on a Cosmosil 5C18 AR-II column (2.0 × 150 mm) with a linear gradient from 0 to 60% acetonitrile containing 0.05% trifluoroacetic acid. The amino acid sequences were determined using a Hewlett Packard G1005A protein sequencing system.

#### Isolation and Sequencing of Genomic DNA Clones Encoding SPT—Based on the amino acid sequences of the SPT peptides, we synthesized degenerate oligonucleotides to obtain partial DNA fragments encoding the SPT gene by PCR against genomic DNA from *S. paucimobilis*. The oligonucleotides were 5′-GA(TC)/GC/TCAG/CC/TCAG/GA(TC)AT(TC)/GA(TC)AT(TC)AG/CC(TC)AG/CG-GG-3′ corresponding to the amino acid sequences DAPDIAP and PY-IPTA, respectively (Fig. 5, dashed lines). The genomic DNA of *S. paucimobilis* was prepared by a standard method (21). PCR was performed using LA Taq DNA polymerase (TaKaRa, Kyoto, Japan) under the following conditions: 30 cycles of 94 °C for 30 s, (40 °C) for 30 s, and 72 °C for 30 s, followed by 10 min. When it was confirmed that the annealing temperature was increased successively by 0.25 °C at each cycle. The PCR product was directly cloned into a pCR\(^\_\)II vector (Invitrogen, Netherlands) and sequenced by the “Dye-Terminator Cycle Sequencing” kit and an ABI 373A DNA sequencer (PerkinElmer Life Sciences). To obtain the full-length SPT gene, a genomic DNA library (1 × 10⁷ recombinants) was screened with the 32P-labeled PCR product (length: 750 bp) as a probe. To isolate the genomic DNA from *S. paucimobilis*, partially digested by *Sau*3AI, 3.5- to 5.5-kilobase fragments were agarose gel-purified and ligated into BamH\_I-digested pUC118, and these constructs were used to transform *E. coli* JM109. Labeling of the probe and detection of hybridized fragments were performed using the BcaBEST\(^\_\)TM labeling kit (TaKaRa, Kyoto, Japan) and Quick-Hyb\(^\_\)TM hybridization solution (Stratagene), respectively. Twelve positive clones of the first screening were isolated. Restriction mapping and partial sequencing of the second screening revealed that all 12 clones were derived from the same gene. The complete DNA sequence was determined for both strands of the three longest clones.

#### Gel Filtration—The enzymes were applied to a Superose\(^\_\)12 and fractionated at a flow rate of 0.5 ml/min with an fast protein liquid chromatography system. Bovine pancreas ribonuclease A (M, 13,700), bovine pancreas chymotrypsinogen A (M, 25,000), hen egg ovalbumin (M, 43,000), bovine serum albumin (M, 67,000), rabbit muscle aldolase (M, 158,000), bovine liver catalase (M, 232,000), horse spleen ferritin (M, 440,000), bovine thyroid thyroglobulin (M, 669,000), E. coli aspartate aminotransferase (M, 43,573 × 2), and E. coli branched-chain amino acid aminotransferase (M, 39,962 × 6) were used as standard proteins.

#### Spectrophotometric Measurement—The absorption spectra of SPT were recorded with a Hitachi spectrophotometer U-3300 at 25 °C. The buffer solution for the absorption measurements contained 50 mM HEPES-NaOH (pH 7.5) and 0.1 mM EDTA. SPT was equilibrated with the above buffer by gel filtration using a PD-10 (Sephadex G-25) column (Amersham Pharmacia Biotech) prior to the measurement.
Other Methods—Protein concentration during the purification procedure was determined with a BCA protein assay kit (Pierce Chemical) using bovine serum albumin as a standard. The protein concentration of purified SPT was determined spectrophotometrically using a molar extinction coefficient of 2.83 $\times 10^4$ M$^{-1}$ cm$^{-1}$ at 280 nm for the PLP form of the enzyme. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with an SDS-Tris system using 10% polyacrylamide gel as a reference; lane 2, crude extract after sonication of $S$. paucimobilis; lane 3, the supernatant after centrifugation at 100,000 $\times g$ of $S$. paucimobilis crude extract; lane 5, crude extract after sonication of $S$. spiritivorum; lane 6, the supernatant after centrifugation at 100,000 $\times g$ of $S$. spiritivorum crude extract.

**RESULTS**

**SPT Activity in $S$. paucimobilis and $S$. spiritivorum—**

$S$. paucimobilis and $S$. spiritivorum contain a large amount of sphingolipids as their cell components (16, 17), and thus sphingolipid biosynthetic enzymes have been expected to exist in these bacteria. Cell-free extracts (100,000 $\times g$ supernatants) prepared by sonication of these strains were examined for SPT activity by incubation with $[14C]$serine and unlabeled palmitoyl-CoA. The lipids were extracted and subjected to TLC analysis. Some radioactive products were detected for each cell fractionation experiment indicates that the SPTs of these bacteria are water-soluble enzymes (Fig. 1). Because the extracts of $S$. paucimobilis showed higher SPT activity than that of $S$. spiritivorum, $S$. paucimobilis was selected as the starting material for the purification of SPT.

**Purification of SPT from $S$. paucimobilis—**

The enzyme was purified to homogeneity by five steps of column chromatography. As shown in Fig. 2, the purified SPT showed a single protein band with an apparent $M_r$ of about 90,000 by gel filtration. Electrospray ionization mass spectrometry (ESI-MS) gave a signal at $m/z$ 44,916. These results show that the native SPT from $S$. paucimobilis has a dimer structure composed of two identical subunits. The purified SPT had an absorption spectrum with two peaks at 338 and 426 nm other than the protein absorption peak at 278 nm in 50 mM HEPES-NaOH (pH 7.5) containing 0.1 mM EDTA (Fig. 3). These absorption peaks are characteristic of PLP enzymes, which contain the cofactor bound to the $\epsilon$-amino group of a lysine residue in the active site (23). The addition of serine to the enzyme gave rise to an intense absorption band at 426 nm and a less intense band at 338 nm, which indicates that 338 nm absorption represents the active species and that the external aldimine complex was formed.

**Catalytic Properties and Substrate Specificity—**

The time course of KDS formation by the purified SPT was almost linear reproducibly over 100% probably because some coexisting inhibitory materials were removed as the purification proceeded. The purified enzyme could be stored at 4 °C in sterile capped vials for up to 2 months in 20 mM Tris-HCl buffer (pH 7.5) without loss of activity.

**Physical Characterizations—**

The $M_r$ of the purified enzyme was estimated to be about 90,000 by gel filtration. Electrospray
methylester by hydrolysis was metabolized to KDS. We also examined the inhibition effect of excess amounts of nonradioactive competitors on the [3H]KDS production from 0.1 mM L-[3H]serine (Table II). [3H]KDS production was inhibited about 80% by 4 mM nonradioactive L-serine under this assay condition. The inhibition of the [3H]KDS production by 4 mM each of other natural amino acids except for cysteine was 40% or less. The effect of cysteine can be ascribed to the thiazolidine formation of cysteine with PLP (23).

O-Phosphoserine was the most effective, with α-methyl-DL-serine, 3-hydroxypropionate, and serine methyester following in this order. Seriamide and serinol were essentially inert. Among various acyl-CoAs examined, palmitoyl-CoA was the best substrate (Table III). The unsaturated bond of the acyl chain of CoA analogs did not significantly influence the SPT activity. The activity chain length profile showed a bell-shaped pattern, which peaked around C16.

Kinetic Parameters of Native SPT— Steady-state analysis of the purified SPT was carried out. The kinetic parameters for SPT were determined with respect to serine and palmitoyl-CoA. As shown in Fig. 4, the experimental data were analyzed according to the ordered Bi-Bi mechanism (24). The $K_m$ values for serine and palmitoyl-CoA were 4.2 and 0.87 mM, respectively, and the $k_{cat}$ value was 140 min⁻¹ (Table IV).

Cloning of the SPT Gene—The nucleotide sequence of one of the three clones sequenced, SPT1 (GenBank™ accession number AB055142), is shown in Fig. 5. SPT1 contains a 1260-base pair open reading frame (65% GC content) encoding a protein of 420 amino acid residues. The amino-terminal protein sequence of purified SPT was Thr-Glu-Ala-Ala-, indicating that the first methionine of purified SPT had been cleaved by processing. The deduced amino acid sequence of SPT is also shown in Fig. 5. The molecular weight of 44,916 obtained by ESI-MS is in

| Competitor       | Relative activity % |
|------------------|---------------------|
| None             | 100                 |
| Serine           | 18                  |
| Glycine          | 96                  |
| Alanine          | 96                  |
| Threonine        | 65                  |
| Cysteine         | 17                  |
| α-Methyl-DL-serine | 23              |
| 3-Hydroxypropionate | 34              |
| Seriamide        | 91                  |
| Serinol          | 98                  |
| Serine methylester | 52                |
| O-Phosphoserine  | 18                  |

FIG. 3. Absorption spectrum of purified SPT. The conditions were as follows: 50 mM HEPES-NaOH buffer, 0.1 mM EDTA, pH 7.5, 25 °C, 0.35 mg/ml enzyme.

![Absorption spectrum of purified SPT](image)

| Substrate               | Relative activity % |
|-------------------------|---------------------|
| Lauroyl-CoA (C12:0)     | 18                  |
| Myristoyl-CoA (C14:0)   | 75                  |
| Palmitoyl-CoA (C16:0)   | 100                 |
| n-Heptadecanoyl-CoA (C17:0) | 75            |
| Stearoyl-CoA (C18:0)    | 51                  |
| Arachidoyl-CoA (C20:0)  | 37                  |
| Myristoleoyl-CoA (C14:1) | 46              |
| Palmitoleoyl-CoA (C16:1) | 80                |
| Oleoyl-CoA (C18:1)      | 57                  |
| Elaidoyl-CoA (C18:1)c   | 39                  |

* cis-9-Octadecenoyl-CoA.
* trans-9-Octadecenoyl-CoA.

**TABLE II**

Effects of various amino acids on the formation of [3H]KDS from L-[3H]serine

Purified SPT (50 ng) was incubated with 0.1 mM L-[3H]serine, 0.8 mM palmitoyl-CoA and 4 mM of each nonradioactive competitor indicated. The levels of radioactivity of [3H]KDS that formed are shown as a percentage of the mean level of [3H]KDS formed in the absence of the competitors. The other natural amino acids were inert as competitors. Each value varied ±10% between experiments.

**TABLE III**

Acyl-CoA specificity of purified SPT

Purified SPT (50 ng) was used. The levels of KDS are shown as a percentage of the mean level of KDS formed with palmitoyl-CoA. Each value varied ±10% between experiments.

**FIG. 4.** Kinetic characterization of native SPT from *S. paucimobilis*. The enzyme assay was performed as described under "Experimental Procedures." [3H]Serine as the substrate and [14C]KDS as the internal standard were used. Panel A, the apparent rate constants ($k_{app}$) for the KDS formation were plotted as a function of palmitoyl-CoA concentration. Each solid line represents the theoretical curve according to the initial velocity equation on the ordered Bi-Bi mechanism using the kinetic parameters summarized in Table IV. Panel B, determination of $K_m$, $K_{cat}$, and $k_{cat}$: Primary plot of [palmitoyl-CoA]/$k_{app}$ versus [palmitoyl-CoA] at various serine concentrations. The secondary substrate (serine) concentrations were 1 mM (closed circle), 2 mM (open circle), 4 mM (closed square), 10 mM (open square), 20 mM (closed triangle), 50 mM (open triangle). Inset, the secondary plot of [palmitoyl CoA]/$k_{app}$ – ordinate intercept replot versus 1/[Ser].
good agreement with the value of 44,910 calculated from the deduced amino acid sequence of SPT without the first methionine within experimental error.

Sequence Comparisons—The non-redundant data bases at the National Center for Biotechnology Information were scanned for amino acid sequences similar to the S. paucimobilis SPT sequence using the BLAST algorithm (25). The predicted S. paucimobilis SPT protein is related to proteins grouped as the α-oxamine synthase family. This gene family includes eukaryotic SPT subunits, 5-aminolevulinic acid synthase in heme biosynthesis, 8-aminolevulinate synthase in biopterin biosynthesis, and 2-amino-3-ketobutyrate-CoA ligase in the threonine utilization pathway, all of which catalyze chemically similar reactions using the cofactor PLP (26–30).

Overall sequence homology is found between these proteins and the S. paucimobilis SPT and mouse LCB1 and LCB2 proteins is shown in Fig. 6.

### Table IV

|                      | $K_m$ (Ser) | $K_m$ (palmitoyl-CoA) | $k_{cat}$ |
|----------------------|-------------|-----------------------|-----------|
| **Native SPT**       | 4.23        | 0.87                  | 140       |
| **Recombinant SPT**  | 10.6        | 0.87                  | 181       |

The predicted S. paucimobilis SPT protein is related to proteins grouped as the α-oxamine synthase family. This gene family includes eukaryotic SPT subunits, 5-aminolevulinic acid synthase in heme biosynthesis, 8-aminolevulinate synthase in biopterin biosynthesis, and 2-amino-3-ketobutyrate-CoA ligase in the threonine utilization pathway, all of which catalyze chemically similar reactions using the cofactor PLP (26–30).

Fig. 5. Nucleotide and deduced amino acid sequences of S. paucimobilis SPT gene. The deduced amino acid sequence is given below the nucleotide sequence. The putative Shine-Dalgarno (SD) sequence is indicated by the double underline. The internal amino acid sequence of SPT determined by Edman degradation is indicated by the underline. The annealing sites of the oligonucleotides for the degenerate PCR cloning are indicated by the dashed underline. An asterisk marks the termination codon. An open circle marks the lysine residue predicted to bind PLP.
except for the NH₂-terminal transmembrane region of mouse LCB proteins. SPT has 27% identity and 48% similarity with mouse LCB1 and 31% identity and 49% similarity with mouse LCB2. Fig. 7 shows the amino acid sequence alignment of S. paucimobilis SPT and three prokaryotic enzymes in the α-oxamine synthase family. There are 33% amino acid identity and 56% similarity between SPT and AONS of Bacillus subtilis, 33% identity and 54% similarity between SPT and 2-amino-3-ketobutyrate-CoA ligase of Bacillus subtilis, and 36% identity and 55% similarity between SPT and 5-aminolevulinic acid synthase of Agrobacterium radiobacter.

Expression of the SPT Gene in E. coli—In order to construct the expression system for S. paucimobilis SPT in E. coli, the internal NdeI restriction site (334ATGCAT) of SPT1 was changed to ATGCAC without changing the codons by site-directed mutagenesis, and the new restriction sites, NdeI and HindIII, were introduced to SPT1 at the translation initiation and termination sites, respectively, by PCR. The modified SPT1 was ligated into a pET21b vector, and the recombinant plasmid was used to transform E. coli BL21(DE3) pLysS cells. The SPT produced was functional, and the product amounted to about 10–20% of the total protein in the crude extract of E. coli. Because of the overproduction of the protein, the expressed SPT would be partially in the apo form, but it could be converted to the holoenzyme by addition of PLP to the cell lysate. The recombinant enzyme was purified to homogeneity using DEAE-Toyopearl, Butyl-Toyopearl, and hydroxyapatite column chromatography. The recombinant SPT provided a 50-kDa band on SDS-PAGE (data not shown). The NH₂-terminal sequence of the purified enzyme, Thr-Glu-Ala-Ala-, agreed with that of the native enzyme. Thus, the first methionine of the recombinant SPT was similarly removed by processing. The purified enzyme showed an absorption spectrum char-

**DISCUSSION**

Because large scale cultivation of strict anaerobic bacteria is difficult and the unsuccessful purification of the B. melaninogenicus SPT has already been reported (15), we searched for aerobic bacteria containing sphingolipids and chose S. paucimobilis as an alternative purification source. S. paucimobilis contains sphingolipids which form more than 30% of the total extractable lipid (16, 19). There is a report that 14C-labeled fatty acids or amino acids were incorporated into the sphingolipids of S. paucimobilis (31). These findings suggest the possibility for this bacterium to contain SPT.

The most important finding is that the S. paucimobilis SPT is water-soluble and is a dimer composed of two identical subunits. All the eukaryotic enzymes examined so far are heterodimers, and both of the subunits are membrane-bound proteins. Membrane localization of eukaryotic SPT complexes seems reasonable because the product of this enzyme is a hydrophobic lipid incorporated into membranes. The relationship between cellular localization and the mechanism of the product release of bacterial SPT is the next subject of research.

How is the reaction product, KDS, transferred to the membrane? Does SPT interact with membrane in vivo? Do other sphingolipid biosynthetic enzymes also exist as water-soluble forms in S. paucimobilis? As for the subunit composition, we can reasonably consider that bacterial SPTs are homodimers. Judging from the high sequence homology between LCB1 and LCB2, ancestral SPT would have been a homodimer, and the gene was duplicated at some point early in the evolution of eukaryotes. The functional benefit for the heterodimerization, or the role of the LCB1 subunit, however, remains unknown.

The purified enzyme showed an absorption spectrum char-
characteristic of the PLP enzyme. The ratio in the peak height of the PLP-derived absorption bands (338 and 426 nm) to the protein-derived band (278 nm) indicates that SPT binds two PLP molecules per dimer.

$S. \text{paucimobilis}$ SPT is not inhibited by halide ions, although SPT activity of $B. \text{melaninogenicus}$ has been reported to be significantly inhibited (15). The inhibition by high concentrations of palmitoyl-CoA, which has been observed for the eukaryotic enzymes, was not detected in $S. \text{paucimobilis}$ SPT (Fig. 4) as well as in the $B. \text{melaninogenicus}$ enzyme (11, 15, 32).

The substrate recognition of $S. \text{paucimobilis}$ SPT was not so strict, especially for the acyl-CoA substrate, compared with the eukaryotic enzymes (11, 32). This observation might reflect the difference in the biological functions of sphingolipids between prokaryote and eukaryote. In eukaryotes, because sphingolipid metabolites take part in the intra- and intercellular signal transduction pathways, it would be necessary to regulate strictly their chemical structures and amounts by the synthetic enzymes. On the other hand, such physiological functions are not known for bacterial sphingolipids.

As shown in Table II, O-phosphoserine inhibited the $[^3H]KDS$ formation from $L-[^3H]$serine as potently as the $L$-serine. This is consistent with the finding that O-phosphoserine was converted to a KDS derivative. Both serinamide and serinol, derivatives in which the carboxyl group of serine is modified, are not potent competitors of $[^3H]$serine in the above reaction. The inhibitory effects of 3-hydroxypropionate and α-methyl-$D,L$-serine agreed with the belief that they can form complexes with SPT, which mimic the Michaelis complex and the external aldimine, respectively. These results indicate that the carboxyl group of serine is essential for the recognition of the amino acid substrates by SPT.

Sequence comparison between the $S. \text{paucimobilis}$ SPT and other prokaryotic enzymes of the α-oxamine synthase family shows that conserved amino acids are distributed throughout the entire sequence (Fig. 7). In addition to Lys-267 that forms a Schiff base linkage with PLP, catalytically important residues identified by x-ray crystallography on AONS from $E. \text{coli}$ are completely conserved at the corresponding positions in SPT, such as His-159, Asp-231, and His-234 (Figs. 6 and 7, asterisk and triangles) (33). These residues interact directly with PLP, and His-159 and Asp-231 are also conserved in other PLP-dependent enzymes such as aspartate aminotransferases from various organisms.

We have succeeded in construction of the overproduction system of SPT in $E. \text{coli}$. The growth rate of $E. \text{coli}$ was not inhibited even after the expression was induced, and the SPT overproduced was catalytically active. Until now, it has been thought that the toxicity of KDS, the reaction product of SPT, is one of the reasons why the expression system of SPT in $E. \text{coli}$ cannot be constructed. However, the present results imply that KDS is not toxic to the $E. \text{coli}$ host, or it is rapidly metabolized. The recombinant SPT was catalytically and spectrophotometrically indistinguishable from the native protein. There has been little success in the overproduction of the enzymes in the sphingolipid biosynthesis pathway, and almost no detailed research exists concerning the enzymatic characterization of these enzymes. This work permitted enough SPT for the three-dimensional structural analysis of this protein, which is essential for elucidation of the reaction mechanism of this enzyme.

We are now attempting crystallization and x-ray diffraction studies of the $S. \text{paucimobilis}$ SPT. Information obtained from the $Sphingomonas$ enzyme would provide us with insight into the more complex eukaryotic homologue.

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S. paucimobilis EY2395 and S. spiritivorum EY3101. We also thank Dr. T. Yano for helpful discussions and critical reading of the manuscript.

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