Membrane Topology of *Escherichia coli* Prolipoprotein Signal Peptidase (Signal Peptidase II)*

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The *isp* gene of *Escherichia coli* encodes the inner membrane enzyme, signal peptidase II (SPase II). SPase II is comprised of 164 amino acid residues and contains four hydrophobic domains. A series of *isp-phoA* and *isp-lacZ* gene fusions have been constructed *in vitro* to determine the topology of SPase II. The fusion junction for each of these gene fusions was determined by DNA sequencing. The lengths of the SPase II fragment in the fusions varied from 12 to 159 amino acid residues. Strains containing SPase II-PhoA fusions to the two predicted periplasmic loops exhibited higher levels of alkaline phosphatase activity than fusions to the predicted cytoplasmic domains. In contrast, SPase II-LacZ fusions at the cytoplasmic and the periplasmic domains of SPase II showed high and low levels of β-galactosidase activity, respectively, a result opposite to those shown by SPase II-PhoA fusions located at precisely the same amino acid of SPase II. Taken together, these results strongly support the predicted model for SPase II topology, i.e. this enzyme spans the cytoplasmic membrane four times with both the amino and the carboxyl termini facing the cytoplasm.

The *Escherichia coli* signal peptidase II (SPase II)1 is an inner membrane protein that cleaves the signal peptide from glyceride-modified prolipoproteins while they are translocated across the cytoplasmic membrane. The structural gene for SPase II (designated *isp*) has been previously cloned, mapped (1, 2), and its nucleotide sequence determined (3, 4). As deduced from the DNA sequence, the SPase II protein is comprised of 164 amino acid residues, lacks an amino-terminal signal sequence, and has a calculated *M* of 18,144 (3). A computer-assisted analysis of its amino acid sequence predicted a highly hydrophobic integral membrane protein, containing four major hydrophobic domains that could span the inner membrane of *E. coli*. In accordance with the "positive inside" rule of von Heijne (5), a working model of the topology of SPase II in *E. coli* cytoplasmic membrane predicts that SPase II contains two periplasmic domains, with both the amino and the carboxyl termini facing the cytoplasm (3) (Fig. 1A).

The present work applies the technique of gene fusions, developed by Beckwith and co-workers (6, 7), to determine the topology of the SPase II in the inner membrane of *E. coli*. A series of gene fusions were constructed between the *phoA* (minus the signal sequence-coding region) and the *isp* gene of *E. coli*. To complement the topological information suggested by the results with *isp-phoA* fusions, some of the *isp-phoA* fusions were converted into corresponding *isp-lacZ* fusions at the same fusion junctions. Our results strongly support the predicted topology of SPase II enzyme as a transmembrane protein spanning the inner membrane four times with both the amino and the carboxyl termini facing the cytoplasm.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids

All fusion plasmids were isolated and maintained in strain CC1168 araD139 Δ(ara-leu)7687 ΔlacX74 galE galK ΔphoA20 thi rpsE rpoB argE(am) recA1) provided by C. Manoil (University of Washington, Seattle, WA) (8). The gene fusions were isolated in plasmid pKM001, that contains the *isp* gene from plasmid pMT521-16 (9) under the control of the inducible lac promoter from plasmid pUC8. Its construction was carried out as follows. Plasmid pMT521-16 was digested with EcoRI and Accl, the *isp*-containing fragment was then inserted into pUC8 that had been previously digested with EcoRI and Accl. A pACYC184 derivative carrying the *lacZ* gene was also transformed into this strain to avoid possible toxic effects caused by high expression of any of the hybrid proteins. Plasmid pRS186 contains a *BamHI·phoA* fragment lacking the signal sequence-encoding region. Its construction was as follows. The *PstI·phoA* DNA fragment from plasmid pCH2 (10) was digested with *BamHI* and *phoA* fragment. The resulting DNA fragment was then inserted into pBR322. Plasmid pMC931 (11) containing a *BamHI·lacZ* fragment was provided by M. Casadaban (University of Chicago, Chicago, IL).

Media and Growth Conditions

Bacterial cultures were routinely grown in L broth at 37 °C. PhoA* or LacZ* colonies were identified on LB plates supplemented with 100 µg/ml ampicillin, 12.5 µg/ml tetracycline, 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and either 40 µg/ml 5-bromo-4-chloro-3-indolyl-phosphate (XP, Sigma) or 40 µg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal, Sigma), respectively.

Recombinant DNA Procedures

Restriction enzyme and exonuclease digestions, Klenow enzyme treatment, ligations, electrophoresis, polymerase chain reaction (PCR), plasmid isolation, and transformations were carried out as described in Sambrook et al. (12). Restriction fragments were isolated and eluted using low melting-point agarose. Oligonucleotide primers for sequencing and PCR were purchased from the Oligonucleotide

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1 The abbreviations used are: SPase II, signal peptidase II; XP, 5-bromo-4-chloro-3-indolyl-phosphate; X-Gal, 5-bromo-4-chloro-3-indolyl-β-D-galactoside; PCR, polymerase chain reaction.

2 *phoA* denotes the structural gene for alkaline phosphatase, and *lacZ* denotes the structural gene for β-galactosidase.
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A.

B.

FIG. 1. Panel A, a proposed model for the topology of SPase II in the inner membrane of E. coli. Charged residues (R, K, H, D, and E) are highlighted. Panel B, a two-dimensional topological model of SPase II in the cytoplasmic membrane of E. coli based on the activities of Lsp-PhoA and Lsp-LacZ fusion proteins. The number in each box or circle denotes the alkaline phosphatase or β-galactosidase activities, respectively. The number below or above the box or circle indicates the number of the last amino acid of SPase II before the fusion. The negatively charged residue in transmembrane segment A (Trp-Ser) corresponds to Asp', and the negatively charged residue in transmembrane segment D (Thr-Ser) corresponds to Glu'.

Synthesis Facility at the Department of Microbiology, Uniformed Services University of the Health Sciences.

Construction of Fusions In Vitro

(i) Plasmids pFM12, pFM23, pFM31, pFM57, pFM65, pFM69, pFM119, pFM138 and pFM151—These fusions were constructed as shown in Fig. 2. Plasmid pKM001 was cleaved either with HindIII, which cuts the plasmid approximately 200 base pairs downstream from the kp gene, or with BssII or XbaI that cut the plasmid just inside that gene. Exonuclease Bal31 was used to remove various amounts of the carboxyl terminus-encoding region of kp. The duplex termini were repaired with Klenow enzyme to generate blunt ends. After addition of BamHI linkers (New England Biolabs, Beverly, MA), the DNA was digested with BamHI and ligated to a phoA fragment obtained from plasmid pRB186 by BamHI digestion. The ligated DNA was used to transform E. coli strain CC118, and Amp' PhoA' transformants were isolated on plates containing ampicillin and XP.

(ii) Plasmid pFM79—An lsp-phoA fusion at the amino acid residue 79 of SPase II was obtained as shown in Fig. 3. An EcoRI-BamHI fragment containing the DNA encoding 79 amino acid residues of SPase II was created by the PCR technique. Two primers were designed as follows. Primer 1 included the EcoRI restriction site that was located approximately 60 base pairs upstream of the lsp gene in pKM001. Primer 2 corresponded to the coding sequence for amino acid residues 73-79 of SPase II and included a BamHI restriction site originally not present in plasmid pKM001. After PCR using pKM001 DNA as the template, the DNA fragment was cloned into a modified pKM001 vector; the latter plasmid was obtained by first converting the HindIII site into a BamHI site and then deleting the resulting EcoRI-BamHI fragment which contains...
the lsp gene. Insertion of the phoA fragment into the BamHI site was then achieved by standard procedures, and the resulting plasmid was transformed into E. coli strain CC118.

(iii) Plasmid pFM159—An lsp-phoA fusion at amino acid residue 159 of SPase II was constructed as shown in Fig. 4. Plasmid pKM001 was cleaved with XbaI, digested with mung bean nuclease and ligated to a BamHI linker. After BamHI digestion, the phoA fragment was inserted into the new BamHI site, and the resulting plasmid was transformed into strain CC118.

(iv) Plasmids pRB93 and pRB106—lsp-lacZ fusions at amino acid residues 93 and 106 of SPase II were obtained by in vitro switching of the phoA fragment in plasmid pRB186 for the original lacZ fragment present at these same locations in plasmids pRB93 and pRB106. Plasmids were isolated, cleaved with BamHI, and electrophoresed on low melting-point agarose to separate the BamHI-lacZ fragment. Isp containing fragments were then recovered and ligated to the BamHI-phoA DNA fragment from plasmid pMC931 to generate the fusion.

(v) Plasmids pFM89 and pFM106—phoA fusions at amino acids 93 and 106 of SPase II were obtained by in vitro switching of the phoA fragment in plasmid pRB186 for the original lacZ fragment present at these same locations in plasmids pRB93 and pRB106. Plasmids were isolated, cleaved with BamHI, and electrophoresed on low melting-point agarose to separate the BamHI-lacZ fragment. Isp containing fragments were then recovered and ligated to the BamHI-phoA DNA fragment from plasmid pMC931 to generate the fusion.

(vi) Plasmid pRB69—An lsp-lacZ fusion at amino acid 69 of SPase II was constructed by in vitro switching of the lacZ fragment for the phoA fragment at the same amino acid residue in plasmid pFM69.

Enzyme Assays
Noninduced overnight cultures of E. coli strain CC118 containing the plasmids described above were used to inoculate 10 ml of fresh medium and grown to an A550 of 0.4. Isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 1 mM, and the culture was incubated for an additional hour. The alkaline phosphatase and β-galactosidase activities were then assayed in permeabilized cells as described by Brickman and Beckwith (13) and Miller (14), respectively.

Western Immunoblotting of the Lsp-PhoA Fusions
E. coli-induced cultures were prepared in the same conditions as described for enzyme assays. Cells were harvested by centrifugation, suspended in 1 ml of 10 mM sodium phosphate buffer, pH 7.0, and sonicated until a cleared lysate was obtained. The samples were lyophilized and suspended in 10 mM sodium phosphate buffer, pH 7.0, containing 1% sodium dodecyl sulfate and incubated at 100 °C for 5 min for the solubilization of membrane proteins. Similar amounts of cell protein were electrophoresed in a sodium dodecyl sulfate-8% polyacrylamide gel. Proteins were electrophoretically transferred onto nitrocellulose paper, and Western immunoblotting with rabbit anti-PhoA antibody (5 Prime-3 Prime, Inc., West Chester, PA) was done essentially as described (15).

The precise fusion junctions were determined by the dideoxy-chain termination method of Sanger et al. (16) as modified by Kraft et al. (17) for double-stranded template sequencing with Sequenase (United States Biochemical Corp., Cleveland, OH). The synthetic oligonucleotide primer (5'-ATCACGCAGAGCGGCAG) that hybridized to the 5' end of the phoA gene was used on the lsp-phoA fusion plasmids. The primer (5'-CGCCACGCTGTGACAAAGG) that hybridized to the 5' end of the lacZ gene was used on the lsp-lacZ fusion plasmids.

RESULTS
Isolation of Randomly Generated lsp-phoA Fusions by Bal31 Exonuclease Deletion—A total of 48 independent blue colonies were initially isolated on XP-containing plates after a 24-h incubation period at 37 °C. Plasmid DNAs were prepared, digested, and screened for the sizes of the lsp-containing EcoRI-BamHI fragments. The mobilities of these fragments in a 5% polyacrylamide gel suggested that all these fusions were probably located in the first periplasmic domain of SPase II according to the proposed topological model (3). This prediction was confirmed by DNA sequencing of the fusion junctions in 10 clones which were selected on the basis of their varying lsp sizes. All the fusions were found to be in-frame, and five of them were selected for further analysis. Plasmids containing these fusions were named pFM23, pFM31, pFM57, pFM65, and pFM69 (Table I).

Four more fusion-containing colonies were isolated on the original transformation plates. Interestingly, these colonies were initially white in color on XP plates but turned into blue-colored colonies after an additional incubation at 4 °C, exactly as reported by Lewis et al. (18) in their topological study of the E. coli F-Fr-ATPase. These fusions were also in frame and located at amino acid residues 119, 134, 138, and 151 of SPase II, respectively, as determined by DNA sequencing. Plasmids containing these fusions were named pFM119, pFM134, pFM138, and pFM151 (Table I).

In order to detect any other possible fusions at different domains of SPase II, restriction analysis of plasmids in 234 colonies that constituted the white background in the transformation plates was also carried out. Among 36 'phoA-containing plasmids, one of them was selected for further study based on the size of its lsp' fragment. DNA sequencing showed that it was an in-frame fusion at amino acid 12 (plasmid pFM12) of SPase II. Five of the other plasmids were also sequenced, all of which contained out-of-frame fusions in the predicted first periplasmic domain.

Construction of lsp-lacZ Gene Fusions—In the first part of this study, we obtained only one fusion in which alkaline phosphatase was attached to a proposed cytoplasmic domain of SPase II (pFM12). More fusions were needed in order to gain a detailed information about the orientation of this enzyme in the cytoplasmic membrane. It has been well established that PhoA fusions to cytoplasmic domains of inner membrane proteins show very low levels of alkaline phosphatase activity leading to white colonies on XP plates that cannot be distinguished from those containing either out-of-frame fusions or no 'phoA insert at all (19). For this reason another strategy was used to isolate additional fusions at the cytoplasmic domains of SPase II. Instead of creating new phoA fusions, we chose to construct lsp-lacZ fusions to locate the cytoplasmic domain of SPase II. Furthermore, by switching 'phoA for lacZ in lsp-lacZ fusions, we could analyze the activities of both alkaline phosphatase and β-galactosidase fused at a particular site in SPase II (8).

lsp-lacZ fusions contained on plasmids pRB93 and pRB106 were isolated on X-Gal-containing plates as blue colonies. The precise location of their fusion joints was determined by
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TABLE I

Location and activity of alkaline phosphatase and β-galactosidase fusions

<table>
<thead>
<tr>
<th>Fusion plasmid</th>
<th>Sequence at fusion junction</th>
<th>Fusion site</th>
<th>Color of colonies on indicator plates</th>
<th>Enzymatic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>lsp-phoA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pFM12</td>
<td>CTA CGC TGG CCG GAT CCG GCT CAG</td>
<td>37/12</td>
<td>White</td>
<td>0.3</td>
</tr>
<tr>
<td>pFM23</td>
<td>ATT ATC GAT CCC GAT CCC GCT CAG</td>
<td>67/23</td>
<td>Blue</td>
<td>45</td>
</tr>
<tr>
<td>pFM31</td>
<td>CTG ATC TGC CCC GAT CCC GCT CAG</td>
<td>94/31</td>
<td>Blue</td>
<td>103</td>
</tr>
<tr>
<td>pFM57</td>
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<td>172/57</td>
<td>Blue</td>
<td>330</td>
</tr>
<tr>
<td>pFM65</td>
<td>GAT AGC GGC GGC GAT CCC GCT CAG</td>
<td>196/65</td>
<td>Blue</td>
<td>149</td>
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<tr>
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<td>208/69</td>
<td>Blue</td>
<td>263</td>
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<td>pFM79</td>
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<td>239/79</td>
<td>Blue</td>
<td>24</td>
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<td>pFM93</td>
<td>TCG AAG GCC AGC GAT CCC GCT CAG</td>
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<tr>
<td>pFM106</td>
<td>ATT ATT GCC GGG GAT CCC GCT CAG</td>
<td>319/106</td>
<td>White</td>
<td>0.5</td>
</tr>
<tr>
<td>pFM119</td>
<td>TGG CAC GCC TGC GAT CCC GCT CAG</td>
<td>358/119</td>
<td>Blue</td>
<td>19</td>
</tr>
<tr>
<td>pFM134</td>
<td>TGG CAC TTC GCC GAT CCC GCT CAG</td>
<td>403/134</td>
<td>Blue</td>
<td>18</td>
</tr>
<tr>
<td>pFM138</td>
<td>ACC ATC AAG CCG GAT CCC GCT CAG</td>
<td>415/138</td>
<td>Blue</td>
<td>19</td>
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<tr>
<td>pFM151</td>
<td>GCC TGC ATT GCG GAT CCC GCT CAG</td>
<td>454/151</td>
<td>Blue</td>
<td>5</td>
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<td>pFM159</td>
<td>TTT TGG CCT TGC GAT CCC GCT CAG</td>
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<td>0.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fusion plasmid</th>
<th>Sequence at fusion junction</th>
<th>Fusion site</th>
<th>Color of colonies on indicator plates</th>
<th>Enzymatic activity</th>
</tr>
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<tbody>
<tr>
<td>lsp-lacZ</td>
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<td>pRB69</td>
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<td>208/69</td>
<td>White</td>
<td>11</td>
</tr>
<tr>
<td>pRB93</td>
<td>TCG AAG GCC ACG GAT CCC GTC GTT</td>
<td>280/93</td>
<td>Blue</td>
<td>662</td>
</tr>
<tr>
<td>pRB106</td>
<td>ATT ATT GCC GGC GAT CCC GTC GTT</td>
<td>319/106</td>
<td>Blue</td>
<td>507</td>
</tr>
</tbody>
</table>

1 The underlined bases indicate lsp sequence and the nonunderlined bases from BamHI linker and phoA or lacZ sequences, respectively.
2 The first number indicates the base number at the fusion junction in the DNA sequence of the lsp gene, and the second number indicates the amino acid residue at the fusion junction in the appropriate sequence.
3 The indicators were XP and X-Gal for PhoA and LacZ, respectively.
4 Expressed in Miller units and assayed as alkaline phosphatase and as β-galactosidase in Lsp-PhoA and Lsp-LacZ fusions, respectively.
5 pFM134 encodes a SPase II-PhoA fusion protein with 135 amino acid residues of SPase II since the fusion junction between the lsp fragment and the BamHI linker encodes Ala which corresponds to the 135th residue of SPase II.

DNA sequencing (Table I). **In vitro** switching of "phoA for lacZ" was then accomplished. DNA sequencing was again performed to ensure that DNA manipulation had not altered the reading frame and that the inserts were in the appropriate orientation.

Construction of lsp-phoA Gene Fusions at Specific Regions of lsp—In order to create a more complete topological map of SPase II, different strategies were necessary to construct phoA fusions in the regions not represented in the original collection. For this purpose, fusions at amino acids 79 and 159 were constructed as described under "Experimental Procedures." The structure of these two gene fusions was verified by DNA sequence analysis.

Enzyme Activities of lsp-phoA and lsp-lacZ Fusions—Alkaline phosphatase and β-galactosidase activities of the fusion-containing strains correlated well with the intensity of blue color on XP and X-Gal plates, respectively (Fig. 1B and Table I). PhoA fusions at amino acids 23, 31, 57, 65, and 69 (predicted to be located at the first periplasmic loop of SPase II) gave higher activities than fusions at amino acids 119, 134, and 138 (predicted to be located at the second periplasmic loop of the protein). On the other hand, PhoA fusions at amino acids 12, 93, and 159, predicted to face the cytoplasm, showed almost no PhoA activities at all (less than 1 unit). Among the fusions to amino acids predicted to be located in the transmembrane segments, fusions at amino acids 79 and 151 exhibited PhoA activities similar to those predicted to face the periplasm, and higher than fusion 106 of which the activity was also less than 1 unit.

As expected, LacZ fusions displayed complementary characteristics to those of PhoA fusions at the same site in SPase II, i.e. high PhoA+ and low LacZ+, and vice versa. LacZ fusions 93 and 106 showed higher activities than fusion 69. The hydrophyt profile (3), the locations of the alkaline phosphatase and β-galactosidase fusions, and their respective enzymatic activities have been used to construct the two-dimensional model shown in Fig. 1B. Also shown in this figure is the distribution of positively (H, K, and R) and negatively charged amino acid residues (D and E) along this protein.

**Western Blot Analysis**—In all cases, the hybrid proteins were examined by Western blotting to ensure that fusion proteins of the expected molecular weights were present. Bands that correlated with the predicted sizes of fusion proteins could be observed in all the strains harboring fusion plasmids. Fig. 5 shows a Western blot of representative lsp-phoA fusions. Some of the fusions showed proteolytic degra-
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We have used the gene fusion technique to study the topology of prolipoprotein signal peptidase (SPase II). A series of isp-phoA gene fusions have been constructed by in vitro techniques. Alkaline phosphatase activities of the fusions have been evaluated, and the hybrid proteins have been detected by Western immunoblotting. Additional information has been provided by β-galactosidase fusions located at the same amino acid residue of SPase II as the PhoA fusions. The properties of both alkaline phosphatase and β-galactosidase fusions to prolipoprotein signal peptidase suggest a membrane topology of this protein identical to that proposed on the basis of its hydrophathy profile (Fig. IB). The low alkaline phosphatase activities exhibited by strains harboring fusions FM12, FM95, and FM159 and the correspondingly high β-galactosidase activity in strain carrying fusion RB93 would place both the NH₂ and the COOH termini as well as the loop connecting transmembrane segments B and C in the cytoplasm. The cytoplasmic location of this loop and the NH₂+COOH-terminal regions is also consistent with the greater frequency of occurrence of positively charged residues in domains exposed to the cytoplasm (5). Further support for this model is provided by the high PhoA activity and low LacZ activity in strains harboring fusion FM69 and RB69, respectively.

While the data of the gene fusions are in excellent agreement with the predicted model, some apparently ambiguous results should be mentioned. PhoA fusions to the first periplasmic domain exhibited much higher alkaline phosphatase activities than those fused to the second periplasmic domain. As suggested in previous studies (18), PhoA fusions may define a periplasmic domain and yet have a low activity if they lack a signal sufficient for efficient export of alkaline phosphatase. Different transmembrane segments of a given inner membrane protein do not promote export across the membrane to a similar extent. Accordingly, the differences in the PhoA activities between fusions at the first and the second periplasmic domains of SPase II should not be used to assign a cytoplasmic location to the latter group. Fusions to those regions predicted to be located in the cytoplasm (fusions 12, 93, and 159) exhibited ≥45-fold lower PhoA activities (Table I) than fusions located at the predicted second periplasmic domain.

Fusions to the predicted transmembrane segments of SPase II exhibited different PhoA activities apparently depending on the orientation of the preceding segment. Thus, while fusions at the second (fusion 79) and fourth (fusion 151) transmembrane segments showed PhoA activities similar to those located at the first and second periplasmic domains, respectively, fusion at the third transmembrane segment (fusion 106) displayed a PhoA activity similar to that of fusion 93 located at the predicted second cytoplasmic domain. We suggest that the incomplete hydrophobic sequences preceding the fusion junctions in these three fusions are not sufficient to anchor the PhoA domain in the cytoplasmic membrane or promote its export across the inner membrane. The fusions to the PhoA or β-galactosidase domains, however, would thus remain in the preceding subcellular compartment. Orientation, relative to the membrane, of the hydrophobic segment immediately preceding the fusion joint may be a very important determinant of the cellular compartment containing the PhoA or LacZ moiety of the fusion proteins with fusion junctions located within a transmembrane segment. These results also support our model for the topology of SPase II, i.e. this enzyme spans the cytoplasmic membrane four times with both the amino and carboxyl termini facing the cytoplasm.

Based on data from fusion protein activities and knowledge of the SPase II hydrophathy profile (3), it is possible to speculate concerning the identities of amino acids comprising the four transmembrane segments (Fig. 1A). Located on either side of the highly-charged Arg₆-Arg₉ region that, on the basis of lacZ fusion activity (Table I), resides in the cytosol, are two long stretches of uncharged and predominantly hydrophobic amino acids that may comprise transmembrane segments B and C. Segment B would include 19 amino acids from Trp²⁷ to Tyr⁶. Segment C would contain 18 amino acids, e.g. Leu⁶-Phe¹₁₃. Both segments would be of sufficient length to span the bilayer in α-helical conformation, particularly if one or more of the charged residues at either end of these segments were part of the helical structure.

Transmembrane segment A probably contains the hydrophobic stretch of 11 amino acids (Trp²⁷-He¹²) and additional COOH-terminal amino acids in the region Asp²⁶-Ser⁸. These four amino acids have been placed in segment A as the resulting 15 residue segment could span the bilayer as a 3₁₀ helix (21). While the placement of aspartic acid within the bilayer interior may be thermodynamically unfavorable, studies of bacteriorhodopsin suggest that charged amino acids may be located in the lipid region (22). For similar reasons we speculate that transmembrane segment D, which is drawn from all the Pseudomonas fluorescens has revealed two structural features of this enzyme which appear to be highly conserved (20). SPase II from all these organisms have a very similar membrane topology with four transmembrane segments. However, these segments show relatively low amino acid sequence homology. Secondly, they contain two regions of high homology: region I (amino acid residues 55-76) with an homology of 86%, and region II (amino acid residues 99-115) with 76%. The homology in these two regions are clearly much higher than the average homology of the rest of these proteins. According to the topological model in Fig. 1B, region I would correspond to the carboxyl-terminal sequence of the first periplasmic domain plus a portion of the second transmembrane segment, while region II would include the third membrane-spanning segment plus a few amino acid residues at the amino-terminal portion of the second periplasmic domain. While more experimental data are needed, it is reasonable to postulate that these two highly conserved regions are good candidates for the catalytic sites of SPase II, whereas the rest of the amino acid residues are less conserved.
acid sequences are required to establish the appropriate topology of the protein in the cytoplasmic membrane.

REFERENCES