somA, a novel gene that encodes a major outer-membrane protein of *Synechococcus* sp. PCC 7942

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The outer membrane of a cyanobacterium (*Synechococcus* sp. strain PCC 7942) contains only a few major proteins. A gene encoding one of them, *somA*, was cloned and characterized. Based on the nucleotide sequence, SomA was predicted to comprise 531 amino acids with a calculated molecular mass of 57 136 Da. The deduced amino acid sequence of SomA shares similarities with two bacterial cell-surface proteins, the S-layer protein of *Thermus thermophilus* and the flagellin of *Campylobacter coli*. The predicted amino acid sequence of SomA revealed also that it contains a signal peptide-like sequence at its N terminus. This signal peptide-like sequence was capable of mediating protein translocation across the cytoplasmic membrane into the outer membrane of *Escherichia coli*, provided that this sequence was fused to the *E. coli* outer-membrane protein, OmpF. The signal peptide-like sequence was cleaved upon the translocation of the SomA::OmpF protein. We suggest that SomA is synthesized as a precursor and that its N-terminal 24 amino acid sequence is a cleavable signal peptide involved in protein targeting into the outer membrane. To our knowledge, this is the first example of cleavable signal peptides for proteins transported into the outer membrane of cyanobacteria.

Keywords: *Synechococcus*, outer membrane, SomA protein, signal peptide, protein targeting

INTRODUCTION

Cyanobacteria can be defined as prokaryotes that have a photosynthetic apparatus similar in structure and function to that of higher plant chloroplasts. Like the majority of Gram-negative eubacteria, cyanobacteria possess an outer membrane as well as a cytoplasmic membrane. In this study, we cloned a gene encoding a major outer-membrane protein (OMP) from one of the most commonly studied unicellular cyanobacteria, *Synechococcus* sp. strain PCC 7942.

We have studied the major OMPs, OmpF and OmpC, in *Escherichia coli* (Mizuno *et al.*, 1983; Mizuno & Mizushima, 1990). These pore-forming proteins (porins) are a paradigm for defining structure and function of the outer membrane in many Gram-negative bacteria. However,

little is known about the structure and function of the outer membrane of cyanobacteria, and as far as we know, there have been no reports on the cloning and characterization of genes encoding OMPs in cyanobacteria.

In cyanobacteria, exported proteins may have to be transported across the photosynthetic membranes (thylakoids) as well as the inner and outer membranes of the envelope. It is well established in *E. coli* that translocation of the majority of protein precursors across the membranes is mediated by the *sec* system (e.g. SecA and SecY) (Pugsley, 1993, and references therein). *Synechococcus* sp. strain PCC 7942 contains the SecA and SecY homologues (Nakai *et al.*, 1992, 1994). Examples of signal peptides have been reported for cyanobacterial secretory precursors, but mostly for proteins transported into the thylakoid lumen (Kuwabara *et al.*, 1987; Philbrick & Zilinskas, 1988; Laudenbach *et al.*, 1990, 1991; Briggs *et al.*, 1990; Chitnis *et al.*, 1991; Zhang *et al.*, 1994; Shestakov *et al.*, 1994). There have been no reports of a signal peptide for cyanobacterial OMPs. Thus, information on the characteristics of signal peptides specific for OMPs is

Abbreviation: OMP, outer-membrane protein.

The EMBL/GenBank/DBJ accession number of the nucleotide sequence reported in this paper is D64077.

needed to understand the molecular mechanism underlying differential protein targeting in cyanobacteria.

METHODS

Bacterial strain and growth conditions. *Synechococcus* sp. strain PCC 7942 was grown at 30 °C in BG11 medium supplemented with 20 mM N-tris(hydroxymethyl)methyl-2-amino-ethanesulphonic acid (TES)-KOH (pH 8.0) (Rippka *et al.*, 1979). For liquid cultivation, the culture was continuously aerated. Continuous illumination was provided by fluorescent lamps. When required, kanamycin was added at a concentration of 30 µg ml⁻¹. *E. coli* K12 MH1160 [*recA* (F⁻ Δ lacU169 *araD139 rpsL relA thiA flbB syrA ompR101 recA*)] was used for expression of the SomA::OmpF fusion protein.

Plasmid construction. Plasmid pUME7 was constructed as follows. A 355 bp *EcoRI* fragment encoding a portion of the *somA* gene was isolated from recombinant λ gt11 phage (see below) and cloned into the *EcoRI* site of pUC118 (Vieira & Messing, 1987). A 5.3 kb *SalI*-*SphI* fragment encompassing the *somA* gene was isolated from the chromosomal DNA of *Synechococcus* sp. PCC 7942 and ligated into the *SalI*-*SphI* site of pKT01 (a derivative of pSC101, unpublished) to yield pHAI182. Plasmid pSomA::OmpF was constructed as follows (see Fig. 6). Firstly, a 616 bp *XbaI*-*EcoRI* fragment, encoding the putative signal peptide of the *somA* gene, was isolated from pHAI182 and ligated into the *XbaI*-*EcoRI* site of pIN-III A3 (Masui *et al.*, 1984) to yield pUME14. A 1.6 kb *PstI* fragment, encoding the *ompF* gene, was isolated from pMAN007 (Matsuyama *et al.*, 1984) and ligated into the *PstI* site of pUC119 (Vieira & Messing, 1987). From the resultant plasmid, a 1.6 kb *HindIII* and *BamHI* fragment was isolated and ligated into the *HindIII*-*BamHI* site of pUME14 to yield pSomA::OmpF.

Construction of deletion/insertion mutant. The *EcoRI*-*EcoRI* region of plasmid pHAI182, which corresponds to an internal region of the *somA* gene, was replaced by a 1.4 kb *EcoRI* fragment of pSI029 containing the *neo* (neomycin phosphotransferase) gene (Suzuki *et al.*, 1987) to yield pUME13. Wild-type *Synechococcus* sp. PCC 7942 was directly transformed by this plasmid, which cannot propagate in the cyanobacterium, and kanamycin-resistant colonies were selected as candidates carrying the *somA::neo* mutation on the chromosome.

DNA techniques. For nucleotide sequence analysis, a set of restriction fragments derived from pHAI182 encompassing the *somA* gene was subcloned into pUC119 (Vieira & Messing, 1987). The generation of successively shortened DNA fragments for nucleotide sequencing was performed using a kilosequence deletion kit (Takara Shuzo). The sequence analyses were performed with a DNA sequencer (Applied Biosystems Model 373A) using a PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). For hybridization experiments, the DIG DNA Labelling and Detection Kit (non-radioactive; Boehringer Mannheim) was used.

Isolation of SomA. *Synechococcus* sp. PCC 7942 was grown in 4 l BG11 medium for 3 d. From 5 g (wet wt) of cells, the envelope fraction was prepared as described by Murata & Omata (1988). This fraction was treated with 1% (w/v) sodium N-lauroyl sarcosinate in buffer containing 10 mM TES-NaOH (pH 7.0), 1 mM EDTA and 10 mM NaCl for 30 min at 20 °C. The insoluble fraction was collected by centrifugation at 100 000 g for 1 h. This fraction was subjected to SDS-PAGE, and Som proteins were purified from the gel by electroelution. This

fraction was used as an antigen to raise a polyclonal anti-Som antiserum in mice.

Screening of a λ gt11 expression library. An expression library, prepared from *Synechococcus* sp. PCC 7942 genomic DNA in λ gt11, was screened using an anti-Som antiserum as a probe. The method was essentially the same as that described by Snyder & Davis (1985). Phosphatase-labelled goat antiserum raised against mouse immunoglobulins was used as an indicator.

Preparation of *E. coli* cell envelopes and analysis by urea-SDS-PAGE. The OMPs of *E. coli* were prepared by extraction of the cell envelope with sodium N-lauroyl sarcosinate as described by Filip *et al.* (1973). The OMPs were characterized by urea-SDS-PAGE as described by Mizuno & Mizushima (1987).

Amino acid sequencing. The SomA::OmpF fusion protein, expressed in the outer membrane of *E. coli*, was separated by urea-SDS-PAGE (see Fig. 7) and transferred onto a PVDF membrane. Amino acid sequencing was performed with an automated amino acid sequencer (Applied Biosystems Model 476A).

RESULTS

Isolation of OMPs

A total cell-membrane fraction, containing the inner, outer and thylakoid membranes, was prepared from *Synechococcus* sp. PCC 7942 (Fig. 1, lane 1). An outer-membrane-enriched fraction was then isolated by means of sucrose density-gradient centrifugation (Murata & Omata, 1988). This fraction was treated with a mild

Fig. 1. SDS-PAGE analysed by Coomassie Brilliant Blue staining (a) or immunoblotting with the anti-Som antiserum (b). Lanes: 1 and 4, total cell envelope proteins, 80 µg protein; 2 and 5, purified OMPs, 8 µg; 3 and 6, purified Som proteins, 3 µg. Note that the purified Som proteins consist of at least two major polypeptides, as indicated by the arrows on the right (see lane 6).

Fig. 2. Schematic representation of the structure of the region containing the *Synechococcus* sp. PCC 7942 *somA* gene. The structure of a λ phage clone is shown at the top in which the *Synechococcus* sp. PCC 7942 *EcoRI*–*EcoRI* DNA segment was inserted in front of the *lacZ* gene. The sequenced region is indicated by the bi-directional arrow, in which an ORF (the putative *somA* gene) was found, as indicated by the bold arrow.

detergent (sodium *N*-lauroyl sarcosinate) and the insoluble protein fraction, presumably containing OMPs, was recovered by centrifugation. Its protein composition was analysed by SDS-PAGE, followed by Coomassie Brilliant Blue staining (Fig. 1a). A major protein band was detected on the gel (lane 2). This band was considered to correspond to major OMP(s) and apparently consisted of only a few polypeptides (tentatively referred to as 'Som', *Synechococcus* outer-membrane proteins). Som proteins were recovered from the polyacrylamide gel by electroelution (lane 3). This Som-enriched fraction was used for preparation of a polyclonal anti-Som antiserum in mice. The same set of protein fractions as those characterized in Fig. 1a were also subjected to an immunoblotting analysis with the resultant polyclonal anti-Som antiserum, (Fig. 1b). The results showed that the anti-Som antiserum recognizes the Som proteins in a highly specific manner (lane 4), and also that the isolated Som fraction appears to contain at least two major polypeptides (lane 6).

Cloning a gene

A λ gt11 expression library in *E. coli*, prepared from *Synechococcus* sp. PCC 7942 genomic DNA, was screened by means of immunoblotting with the anti-Som antiserum. From among 5×10^5 phages, nine positive clones were isolated. Restriction mapping and Southern hybridization analyses of these phage DNAs indicated that they each carried an overlapping DNA segment, but of different length. One of the clones was found to carry a 350 bp *EcoRI*–*EcoRI* fragment of *Synechococcus* sp. PCC 7942 DNA, which was shared among all of the other clones (Fig. 2). The nucleotide sequence of this *EcoRI* fragment revealed a potential ORF fused in-frame to the coding sequence of β -galactosidase derived from the expression vector (Fig. 2). This implied that the cloned DNA encompasses only a portion of the putative *som* gene. Therefore, total genomic DNA from *Synechococcus* sp. PCC 7942, digested with various restriction enzymes, was extensively analysed by Southern hybridization with the *EcoRI* fragment as a probe. A 5.3 kb *SalI*–*SphI*

fragment of *Synechococcus* sp. PCC 7942 DNA, which hybridized with the *EcoRI* probe, was subsequently cloned to yield plasmid pHAI182 (see Methods). This fragment was confirmed to contain the *EcoRI*–*EcoRI* region in question (Fig. 2). It should be noted here that we identified only this type of clone during this screening. Although the anti-Som antiserum appeared to cross-react with two distinct major polypeptides, we did not detect another DNA sequence (see Fig. 1b). We assumed that the cloned *SalI*–*SphI* region may contain the coding sequence for one of the *Synechococcus* sp. PCC 7942 major OMPs.

Nucleotide sequence of the *somA* gene

The determined nucleotide sequence of the approximately 2 kb region surrounding the *EcoRI* sites revealed an ORF encoding 531 amino acids, which is preceded by a good prokaryotic ribosome binding site (GAGG) (Fig. 3). A potential transcription start site was determined by primer extension analysis (underlined G residue at position 195 in Fig. 3). A pair of prokaryotic canonical promoter sequences (–35 and –10 regions) were tentatively assigned as TAGTTT and TACCTT. However, the N-terminal amino acid sequence of the purified Som protein could not be determined, probably because of a modification. Thus, both the identity of this ORF and its initial methionine could not be verified.

The putative *somA* gene was disrupted in the chromosome of *Synechococcus* sp. PCC 7942 by gene replacement using an insertionally inactivated *somA::neo* gene (Fig. 4a, see Methods). Homologous double recombination in the chromosome was confirmed by Southern hybridization with the *SalI*-digested DNA (Fig. 4b). The cell envelope fraction was isolated from the *somA::neo* deletion/insertion mutant, and its protein composition was compared with that of the parental strain by SDS-PAGE (Fig. 4c). These results showed that the mutant strain lacks a major protein in the envelope (SomA), although it still contains another one (named SomB) encoded by a separate gene.

Features of the predicted amino acid sequence for SomA

As described above, the translation start site could not be experimentally identified for SomA. However, the amino acid sequence of SomA was predicted to comprise 531 residues with a calculated molecular mass of 57136 Da (Fig. 3). This value is slightly larger than that estimated for the isolated SomA protein on SDS-PAGE (i.e. 55 kDa). In this respect, it should be pointed out that the predicted amino acid sequence contains a signal peptide-like sequence at its N terminus (see Fig. 3, underlined sequence). There is a hydrophobic core sequence preceded by positively charged amino acids (Lys and Arg) followed by amino acids with no or small side chains (Gly and Ala). All these characteristics are consistent with those proposed generally for bacterial signal peptides (von Heijne, 1994). If this is the case, then the difference between the estimated and calculated molecular masses may be reason-

<u>Xba I</u>	
TCTAGAGCTACGGCACTAGTTAGTTAATCCTTGGGAGAGGGTGGGGTCCGCAAGATCTCACCTCTCTGTTTTGAGGGAAGCTGGGTTT	90
TGGGTGACCTCCTTGAGAGAAGTGTGCAAGTTTTGCAAAACGTTATCGCAGGATGTGTTCCAGAAGACTAGTTTTTTGACAAGGGATT	180
-35	
GTTACCTTACCTTCGTGAGAAGTGATCTAGCCATTTCTCCCTACATGGTGTGAGGTTATCAATGAAACGCCTTTCTCGGCGCTGCTTCT	270
-10	10
AGCCCGGCAATTGCTGGTGTGGCTGCGGGCGCAGCAAAATGCTAACGGTTTAAGCACCGAACAGCTCCAAAAAATTGACGCTGTAAGTCC	360
A P A I A G V A A G A A N A ∇ N G L S T E Q L Q K I D A V T P	40
CAACGGCATCACCTCCGGTCAGATCACTTCGATCACCGAATTGAGTGATGTCAAGCCGACTGACTGGGCTTATCAAGCATTGCAGTCGCT	450
N G I T S G Q I T S I T E L S D V K P T D W A Y Q A L Q S L	70
AGTTGAGCGTTATGGCTGCATCGTCGGCTACCTGATCGGACTTACCGTGGTAGCCGCCCTCTCTCGTTATGAGTTTGCAGCAGGCTT	540
V E R Y G C I V G Y P D R T Y R G S R P L S R Y E F A A G L	100
<u>EcoR I</u>	
GAACGCTTGCTTGGACAAAGTCATTGAATTTGCAGCGTCGAAAGAGGATCTCGACACCTCAAGCGACTGACTGAAGAATTCAGAGCTGA	630
N A C L D K V I E F A A S K E D L D T L K R L T E E F Q A E	130
GCTGGCGACCTGCGTGGTGTGTTGATAGTCTCGAGGCTCGTGTAAAGAGCTCGAAGCTACCGTTTCTCCACCAGGACTAAGCTACA	720
L A T L R G R V D S L E A R V K E L E A T R F S T T T K L Q	160
AGGTGAAGTAATCTTCAGCTTGGACGCTGTTGCTAATACTGCTGGCAACGAAGTAACCAAGCGGTGCTGATCGTTGCGCAACCGCT	810
G E V I F S L D A V A N T A G N E R N Q D G A V S F G N R V	190
CAGCTTGAACCTCAACAGGAGCTTCACTGGCAAGACTTGTATTGACCGCTTGCAGCGCTCGTAACATTGAGACGATTGACGAGCGCTT	900
S L N L N T S F T G K D L L L T R L R A R N I E T I' Q Q R L	220
<u>EcoR I</u>	
GTCCGCTGGCTTTAATCCCTCGGGCTCGCGCTCGACTACGACGGTACCGGTTCCGCTGGTGTCCGGAATTCGGCTAATACCTTTTCTCT	990
S P G F N P S G S R L D Y D G T G S P G V P N S A N T F P L	250
TGACAAATTGCTGTACCGCTTCCCGTCGGTGATGTCTCTTCACTGTTGGTACCGGGCGGTTCAACCTCAAGACTACGGCTGAGCGA	1080
D K L L Y R F P V G D V S F T V G T A G V Q P Q D Y G L S D	280
CGCTACCTTCTTCAGTGGCCCTGCGAACGAAAGCCTTCAAGTATGTCGGTGCAGGTGTTTACGCTGATACTCGTGATGCTGATACCGC	1170
A T F F S G P A N T K A F K Y V G A G V Y A D T R D A D T A	310
TGGTGTGGCTTCAACTGGAAGGCTAGTAAGAATTTCACTTCCAGGCTGGCTACATCAACCGCAACTCTGCTGATGTCTCGACTGTCAA	1260
G V G F N W K A S K N F S F Q A G Y I N R N S A D V S T V N	340
CAGTGGTGGTGTCTTTGGCTTACCCCGACAGGACTGGTACTAATCTTGGGATGTGAATGCTCAAGTCAAGTACCAAACTGATAACAA	1350
S G G V F G F T P T G T G T N S W D V N A Q V K Y Q T D N N	370
CAAGTCCGAGTGTCTTGGCTACGCTCTGCGAAATGGTCTACGCCACTGACTTCGGTACCACCAACGCCCTTCCAGCCTTTTGGTGC	1440
K F R V A L A Y A L R N G R Y A T D F G T T N A F Q P F G A	400
TACCAACTACAACAGCAACAACCTTGCTCTCTCTTGGGTTGGGCAATTTCTGATGTGTGACCTTGTGAGCCGGCTACGGCATTTGGCTT	1530
T N Y N S N N L A L S L G W A I S D A V T L S A G Y G I G F	430
TGTAATGAGCAAGGTACGAACCAACCGGACTGTTGAGAACTACGCAATTGGCTTACCTTCCCTAACCTGTTTGTGATGGCAACGA	1620
V N E Q G T N Q N A T V Q N Y A I G L T F P N L F A D G N E	460
GTTTGGTGTGACGAGGCCAACCACTTGGGTGAGCAGCGCTTCAACCGTTCGTCTGAAGCACTGGTTCCTTTGGTGTGAAACCTA	1710
F G V A A G Q Q P W V S S A S N R S S E D T G S F G V E T Y	490
CTACAAATCCAAGTGACGGACAATATCTCCATCACTCTGGTATTACGTGTTCAACAACCAATGGCCAGCAAAACGGTGGAAACGAC	1800
Y K F Q V T D N I S I T P G I Y V F N N T N G Q Q N G G T T	520
TTACGTTCCCTTCTGAAAACAGTCTTCCGTTTCTAGGAATCAACTGTTTAGTTAGGGATGGGCGATCGCATCTAACCAACTCTTTCCT	1890
Y V P F L K T V F R F *	531
CACCACATTAGCTCTCGCTGTCGAGAGCTTTTTTATGCTTTTT	1933

Fig. 3. The nucleotide and deduced amino acid sequences of the *somA* gene. The transcription start site of *somA*, determined by primer extension analysis, is indicated by the underlined G at position 195. A signal peptide-like sequence is underlined and a possible cleavage site is indicated by ∇ .

ably explained by assuming that the *somA* gene specifies a precursor.

A computer-aided search (using the PIR and Swiss-Prot databases, provided by the DDBJ e-mail server) revealed that SomA shares certain similarities with two other known bacterial proteins, although each similar region is located at different and limited portions of SomA (Fig. 5). A 90 amino acid sequence of SomA (positions 50–140)

appears to be significantly similar to a portion of the S-layer protein of *Thermus thermophilus* (Fig. 5b) (Faraldo *et al.*, 1992). This N-terminal region of SomA is followed by a 200 amino acid region (positions 140–340), which resembles a portion of the flagellin (FlaA) of *Campylobacter coli* (Fig. 5c) (Guerry *et al.*, 1991). It should also be noted that a more extensive similarity was found between SomA and a hypothetical protein predicted previously in another cyanobacterium, *Synechocystis* sp. strain PCC 6803 (data

Fig. 4. (a) Restriction map of the *Synechococcus* sp. PCC 7942 genomic DNA encompassing the *somA* gene, and isolation of an insertional inactivation mutant. To construct a *somA::neo* deletion/insertion mutant (designated as Δ *somA*), the *neo* (kanamycin resistance) gene was inserted between the *EcoRI* sites as described in Methods. (b) Southern hybridization analysis of the chromosomal DNA from the *somA* deletion (lane 2) and wild-type cells (lane 1) was carried out with the *SalI*-*EcoRI* segment shown in (a) as the probe. (c) SDS-PAGE of the cell envelope proteins analysed by Coomassie Brilliant Blue staining (lanes 1 and 2) or immunoblotting (lanes 3 and 4), prepared from the *somA* deletion (lanes 2 and 4) and wild-type cells (lanes 1 and 3).

not shown) (Zhang *et al.*, 1989). However, nothing is known about this hypothetical protein.

The signal peptide-like sequence of SomA mediates protein translocation into the outer membrane of *E. coli*

As mentioned above, it was not possible to demonstrate that SomA has a cleavable signal peptide. Thus we addressed this issue using a model prokaryotic species, *E. coli*. *E. coli* K12 possesses the well-characterized OmpF which is synthesized as a precursor with a typical cleavable signal peptide (Fig. 6a) (Mizuno *et al.*, 1983). We constructed a *somA::ompF* fusion gene which could specify a SomA::OmpF fusion protein, in which an N-terminal portion of SomA was fused to the entire region of the mature OmpF protein (Fig. 6a). This particular fusion gene was constructed on an *E. coli* expression vector (plasmid pSomA::OmpF, see Methods). This plasmid was introduced into an *E. coli* mutant lacking OmpF and its expression was induced under the *lac-lpp* promoter of the plasmid. OMPs were purified from the *E. coli* cells and analysed by SDS-PAGE (Fig. 6b). A large amount of SomA::OmpF fusion protein was synthesized and translocated into the *E. coli* outer membrane (lanes + in Fig. 6b; note that in the immunoblotting analysis the smaller band appears to be a degradation product of SomA::OmpF). It is known that OmpF in the *E. coli*

Fig. 5. Characteristic features of the primary structure of SomA. (a) A schematic representation of the structure of SomA. The signal peptide-like sequence (■) is followed by a region similar to the S-layer protein of *Thermus aquaticus* (□) for the sequence comparison, see (b) (□). This S-layer-protein-like region is followed by a region similar to the flagellin (FlaA) of *C. coli* (for sequence comparison, see (c) (▨)). In (b) and (c), the respective sequences were aligned using the FASTA program. Identical and similar amino acids are highlighted by asterisks and dots, respectively.

outer membrane functions as an essential receptor for phage Tu-1a (Datta & Henning, 1977). *E. coli* cells producing the SomA::OmpF fusion protein supported the propagation of Tu-1a, while the OmpF⁻ strain did not (Fig. 6c). This result indicated that the SomA::OmpF fusion protein can be properly incorporated into the outer membrane, thereby functioning as the receptor for Tu-1a. The SomA::OmpF fusion protein was purified and subjected to N-terminal amino acid sequencing. The deduced sequence of Asn-Gly-Leu-Ser-Thr-Glu corresponds exactly to the amino acid sequence found in the near N terminus of SomA, extending from positions 25–30 (Fig. 6d). Collectively, these results imply that the N-terminal 24 amino acid sequence of SomA can function as a cleavable signal peptide in *E. coli*. We believe that this is most likely the case also in *Synechococcus* sp. PCC 7942.

Fig. 6. Characterization of a *somA::ompF* translational fusion gene encoding a SomA::OmpF fusion protein in *E. coli*. (a) Structure of the SomA::OmpF protein. The approximate location of the sites at which the SomA and OmpF proteins were fused together are shown by the arrow. (b) SDS-PAGE analysed by Coomassie Brilliant Blue staining or immunoblotting with a monoclonal anti-OmpF antiserum. *E. coli* OMPs were purified from the cells producing the SomA::OmpF protein and then analysed by SDS-PAGE (lanes +) by comparison with the sample from the control cells (OmpF⁻, lanes -). (c) A cross-streak test with *E. coli* phage Tu-1a. A lysate of Tu-1a was pre-streaked on plates, then either the *E. coli* cells producing the SomA::OmpF protein or the control cells (OmpF⁻) were cross-streaked against it. The plates were incubated at 37 °C overnight, and then photographed. (d) The putative signal sequence of SomA. The cleavage site was determined for the isolated SomA::OmpF protein (see b; the band corresponding to SomA::OmpF was isolated from the gel by electroelution. The sample was then directly analysed by an automated amino acid sequencer. Based on this result, the cleavage site was determined, as indicated by the arrowhead.

DISCUSSION

This study is the first report of the cloning and sequencing of a gene (*somA*) encoding a major OMP of the unicellular cyanobacterium *Synechococcus* sp. PCC 7942. SomA is one of the most abundant proteins of the total envelope proteins of *Synechococcus* sp. PCC 7942 (see Fig. 1, lane 1). The physiological function of SomA is not known at present, since we have not been able to detect any noticeable phenotype for a *somA::neo* deletion/insertion mutant under the growth conditions tested (see Fig. 4). *E. coli* K12 has three major OMPs, OmpA, OmpC and OmpF, each numbering approximately 10^5 to 10^6 molecules per cell. Both OmpC and OmpF play an important physiological role by forming voltage-gated passive diffusion pores (porin) which allow small molecular weight hydrophilic materials to cross the outer membrane

(Nikaido, 1979). With regard to its primary sequence, SomA is not similar to the OmpC and OmpF proteins. Voltage-gated porins are also known to exist in plant chloroplasts and their predicted sequences have recently been reported for non-green plastids from pea and maize (Fischer *et al.*, 1994), but the reported amino acid sequences are not similar to that of SomA. Recently, a pore-forming protein was purified from the outer membrane of *Synechococcus* sp. PCC 6301 (Hansel *et al.*, 1994). The apparent molecular mass of this protein was estimated by SDS-PAGE to be about 52000 Da, similar to that of SomA. It is not known at present whether or not these two proteins from different *Synechococcus* species are homologous. In any case, it may be worth mentioning that *Synechococcus* sp. PCC 7942 has another major OMP (SomB) which is encoded by a gene distinct from *somA* (see Fig. 4).

While the physiological function of SomA is not known at present, it shares certain similarities with two proteins, the cell-surface S-layer protein of *T. thermophilus* and the flagellin of *C. coli* (see Fig. 5). In particular, the polymeric S-layer protein appears to play a structural role. By this analogy, SomA may play a structural role by forming a protein layer on the cell surface.

Some of the thylakoid proteins in cyanobacteria must be transported completely across the thylakoid membrane into the thylakoid lumen, whereas cell envelope proteins must be transported across the cytoplasmic membrane into either the periplasmic space or the outer membrane. A crucial question is the mechanism responsible for the ability of cyanobacteria to discriminate between these two pathways for proper protein targeting. Are there two distinct types of translocation machinery in cyanobacteria, or are there two types of signal peptides for secretory precursors, one specific for the thylakoid lumen proteins and the other for the cell envelope proteins? Although several recent studies have addressed these questions, a clear answer has not emerged (Nakai *et al.*, 1992, 1994; Barbrook *et al.*, 1993; Mackle & Zilinskas, 1994; Chaddock *et al.*, 1995; Varley *et al.*, 1995; Packer *et al.*, 1995). The results implied that the cytoplasmic and thylakoid membranes share a common protein translocation machinery, which includes Sec proteins (e.g. SecA and SecY) as well as signal peptide peptidases (Kuwabara *et al.*, 1987; Philbrick & Zilinskas, 1988; Laudenbach *et al.*, 1990; Briggs *et al.*, 1990; Chitnis *et al.*, 1991; Zhang *et al.*, 1994; Shestakov *et al.*, 1994). In this study, we compiled a list of putative signal peptide sequences for comparison to SomA; these include the periplasmic protein (RhdA, a rhodanese-like protein of *Synechococcus*) (Laudenbach *et al.*, 1991), as well as a number of thylakoid lumen proteins (Fig. 7). The listed signal peptides all resemble each other with respect to their structural features. Specifically, they contain a positively charged N-terminal domain, a hydrophobic core sequence and a conserved signal peptide cleavage site. These are well-known characteristics for eubacterial signal peptides (von Heijne, 1994). This observation explains the basis that the SomA signal peptide is capable of functioning in the translocation of OmpF in *E. coli* (see Fig. 6). It should be emphasized that

SomA (<i>Synechococcus</i>) (OM):	MKR	LFSALLAPAIAGVAA	GAANA	ngl
RhdA (<i>Synechococcus</i>) (PS):	MSVRSRLWRPRQK	AFLAVISLVVAVLLAVPGWL	TPATA	asq
33 kDa (<i>Synechococcus</i>) (TL):	MYR	AFLAAFLAVCLGVLTA	GPTAA	dlg
CytA (<i>Synechococcus</i>) (TL):	MKR	ILGTAIAALVLLAFI	APAQA	adl
33 kDa (<i>Synechocystis</i>) (TL):	MRFR	PSIVALLSVCFGLLTFLYS	GSAFA	vdK
PetE (<i>Synechocystis</i>) (TL):	MSKK	FLTILAGLLLVSSFFLSVS	PAAAA	nat
PetJ (<i>Synechocystis</i>) (TL):	MFKLFNQASR	IFFGIALPCLIFLGGIFSLG	NTALA	adl
PsaF (<i>Synechocystis</i>) (TL):	MKHKK	ALLAFTLWFNFA	PSASA	ddf
CtpA (<i>Synechocystis</i>) (TL):	MGRKTRR	FWALAFSLMLGALIYLGNT	PSALA	fte

Fig. 7. Comparison of the primary structures of cyanobacterial signal peptides. The SomA OMP (OM) was characterized in this study. The RhdA protein has been reported to be transported into the periplasmic space (PS). All others are known to be transported into the thylakoid lumen (TL). In each sequence, for clarity, a positively charged N-terminal domain (left), a hydrophobic core sequence (centre) and a conserved signal peptide cleavage site (right, arrowhead) are presented separately. The lower case letters for each sequence indicate the presumed N-terminal sequence of the corresponding mature form. These sequences (from top to bottom) were from those reported by Laudenbach et al. (1991, 1990), Kuwabara et al. (1987), Philbrick & Zilinskas (1988), Briggs et al. (1990), Zhang et al. (1994), Chitnis et al. (1991) and Shestakov et al. (1994).

the SomA signal peptide appears indistinguishable from, for instance, that of the thylakoid lumen protein, CytA (cytochrome *c*-553) (Fig. 7). These comparisons imply that protein targeting in cyanobacteria may not be achieved by radical differences in signal peptide structure.

ACKNOWLEDGEMENTS

We thank Drs N. Murata and T. Omata for helpful discussions. This study was supported by a Grant-in-Aid for Scientific Research on a priority area (No. 04273013) from the Ministry of Education, Science and Culture of Japan.

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Received 17 November 1995; revised 29 February 1996; accepted 18 March 1996.