Functional characterization of the *Staphylococcus carnosus* SecA protein in *Escherichia coli* and *Bacillus subtilis* secA mutant strains

Michael Klein, Jochen Meens, Roland Freudl *

Institut für Biotechnologie I, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany

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Abstract

The *Staphylococcus carnosus* secA gene was cloned using the *Bacillus subtilis* secA gene as a probe. The *S. carnosus* secA encodes a polypeptide of 844 amino acid residues which is homologous to other known SecA proteins. The *S. carnosus* SecA functionally complemented the growth and secretion defects of a temperature-sensitive *B. subtilis* secA mutant at the non-permissive temperature. In contrast, the growth defect of an *Escherichia coli* secA mutant could not be complemented by the *S. carnosus* SecA protein. Our results suggest that the interactions of SecA with precursor proteins and/or other components of bacterial preprotein translocase are optimized within each organism.

Keywords: *Staphylococcus carnosus*; Protein translocation; SecA protein; Preprotein translocase

1. Introduction

In *Escherichia coli*, the translocation of proteins across the plasma membrane is mediated by preprotein translocase, a multi-subunit complex consisting of the integral membrane proteins SecY, SecE, SecG and the peripheral subunit SecA [1]. The SecA protein [2] has been shown to initiate the translocation of precursor proteins by undergoing ATP-driven cycles of membrane insertion and de-insertion [3], after which further translocation might mainly be driven by the protonmotive force [4].

Gram-positive bacteria can secrete large amounts of proteins directly into the growth medium and are considered to be attractive as potential host organisms for the secretory production of homologous and heterologous proteins. In the last few years, substantial progress has been made in the elucidation of the mechanism of protein transport in these microorganisms [5]. Homologues of SecA [6,7], SecY [8,9], and SecE [10] have been identified in *Bacillus subtilis*, demonstrating that the components of preprotein translocase and, most likely, the mechanism of protein translocation are highly conserved in eubacteria.

In contrast to most *Bacillus* species, the Gram-positive bacterium *Staphylococcus carnosus* is mainly devoid of extracellular proteases [11]. Therefore, *S. carnosus* seems to be a promising host for the secretory production of protease-labile, heterologous proteins and detailed knowledge of the mechanism of protein translocation in this organism would be desirable. So far, the integral membrane components

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* Corresponding author. Tel.: +49 (2461) 613 472; Fax: +49 (2461) 612 710; E-mail: freudl@ibt013.ibt.kfa-juelich.de.
SecY [12] and SecE [13] of the S. carnosus preprotei
n translocase have been identified. In this communi-
cation we describe the cloning and nucleotide se-
quence of the S. carnosus secA gene. In addition, the
 corresponding S. carnosus SecA protein was ex-
 pressed and functionally characterized in E. coli and
B. subtilis secA mutant strains.

2. Materials and methods

2.1. Bacterial strains and growth conditions

E. coli SURE™ (Stratagene) and MM66
(geneX™, supF™) [14] were grown in LB medium
[15] supplemented with 50 μg ml⁻¹ ampicillin, 30
μg ml⁻¹ chloramphenicol, 0.5% (w/v) glucose, or 1
mM IPTG, as required. B. subtilis NIG1152
(dw431™) [16] was grown in LB or S7 minimal
medium [17] supplemented with 15 μg ml⁻¹ chlo-
ramphenicol, 15 μg ml⁻¹ tetracycline, 0.5% (w/v)
glucose, or 0.5% (w/v) xylose, as required. S.
carnosus TM300 [11] was grown in LB medium.

2.2. DNA techniques

Isolation of chromosomal DNA, Southern hy-
bridization and other DNA techniques followed stan-
dard procedures [18]. Plasmid pMA10, containing
the S. carnosus secA gene, was obtained by cloning
with the PC/Gene software package (IntelliGenetics
Inc., Mountain View, CA) into pHSG576 [19] and, after transformation into
SURE™, by subsequent screening of the resulting
transformants by colony hybridization using the B.
was obtained by cloning a 4-kb HpaI/PstI DNA
fragment from pMA10, harboring a promoterless S.
carnosus secA gene, into E. coli expression vector
pTRC99A [21]. Plasmids pMKL18 (containing the
E. coli secA gene) and pMKL40 (containing the
B. subtilis secA gene) have been described previously
[20]. For the expression of the secA genes in B.
subtilis, the corresponding promoterless secA genes
were subcloned into B. subtilis expression vector
pWH1520 [22]. Plasmid pWA11 (containing the S.
carnosus secA gene) was obtained by cloning a
2.6-kb BstXI/SphI DNA fragment from pMA12
into pWH1520. pWE10 (containing the E. coli secA
gen) was constructed by cloning a 2.7-kb
NdeI/XbaI DNA fragment of pMKL18 into
pWH1520. Plasmid pWMKL1 (containing the B.
subtilis secA gene) has been described previously
[23].

2.3. Miscellaneous techniques

Western blotting and pulse–chase experiments in
B. subtilis were done as described previously [17].
Nucleotide and amino acid sequences were analyzed
with the PC/Gene software package (IntelliGenetics
Inc., Mountain View, CA).

<p>| Table 1 |
| Degree of relatedness of SecA proteins from various organisms |</p>
<table>
<thead>
<tr>
<th>Sc</th>
<th>Ec</th>
<th>Bs</th>
<th>Cc</th>
<th>Ss</th>
<th>Lm</th>
<th>Pl</th>
<th>Ol</th>
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<tr>
<td>Sc</td>
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<td>73.2</td>
<td>57.7</td>
<td>56.7</td>
<td>73.2</td>
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<td>50.0</td>
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<td>64.9</td>
<td>64.9</td>
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<td>79.7</td>
<td>54.1</td>
<td>52.2</td>
<td>54.7</td>
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<td>51.5</td>
<td>47.0</td>
<td>53.8</td>
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<tr>
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<td>60.2</td>
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<td>38.0</td>
<td>39.8</td>
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<td>40.2</td>
<td>46.9</td>
<td>42.7</td>
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Values below the diagonal represent percentage of identical amino acid residues; values above the diagonal represent percentage of identical
plus chemically related amino acids. The SecA proteins are from Staphylococcus carnosus (Sc; X79725), Escherichia coli (Ec; M20791),
Bacillus subtilis (Bs; D10279, D90218), Caulobacter crescentus (Cc; U06928), Synechococcus sp. (Ss; X74592), Listeria monocytogenes
(Lm; L32090), Pseudomonas putida (Pl; X65961), Olisthodiscus luteus (Ol; Z35718), and Antithamnion sp. (At; X64705). The numbers
indicated in parentheses represent the accession numbers of the corresponding nucleotide sequences in the EMBL data library.
Fig. 1. Nucleotide and predicted amino acid sequences of a 3.7-kb DNA fragment from the S. carnosus secA region. Nucleotides are numbered from the 5' end. The putative ribosome-binding sites (RBS) and a putative terminator region are underlined. The sequence data reported in this paper appear in the EMBL Nucleotide Sequence Data Library under accession number X79725.
3. Results

3.1. Cloning and nucleotide sequence of the S. carnosus secA gene

Chromosomal DNA of S. carnosus was digested with various restriction enzymes and Southern blotting was performed using the B. subtilis secA gene [20] as a probe. In PstI-digested DNA, a single 8.5-kb DNA fragment gave rise to a positive hybridization signal. For the cloning of this fragment, chromosomal DNA of S. carnosus was digested with PstI and fragments, 7-10 kb in size, were isolated and cloned into plasmid pHSG576 [19]. Screening of this partial gene library with the B. subtilis secA gene by colony hybridization resulted in the isolation of plasmid pMA10, which contained a PstI insert of 8.5 kb. The nucleotide sequence of a 3.7-kb DNA fragment from pMA10, encompassing the S. carnosus secA gene is shown in Fig. 1.

Two open reading frames (ORFs) were identified on this DNA fragment. The second ORF (ORF2) starts with an ATG codon at position 1012 and encodes a putative protein of 844 amino acid residues. Upstream of ORF2, a putative ribosome binding site (AGGAG) at positions 999–1003 can be identified. Downstream of ORF2, sequences are found between positions 3558–3646 which might constitute a rho-independent terminator. Computer analysis of the deduced amino acid sequence revealed that ORF2 is highly homologous to other known SecA proteins (Table 1), strongly suggesting that we indeed have cloned the S. carnosus secA homologue. The ORF which is located upstream of the S. carnosus secA gene (ORF1; positions 56–619) was found to be homologous to a B. subtilis gene (ORF189) which, also in this case, is located upstream of the secA gene [24] and which might be involved in the regulation of σ^54 activity [25].

3.2. The S. carnosus SecA protein does not complement the growth defect of a conditional-lethal E. coli secA mutant

For the functional analysis of the S. carnosus SecA in the temperature-sensitive E. coli secA mutant MM66 (geneX^m, supF^r) [14], the S. carnosus secA gene was subcloned into the expression vector pTRC99A [21] under the regulatory control of the trc-promoter/lac-operator, resulting in plasmid pMA12. Plasmids pMKL18 (containing the E. coli secA gene) and pMKL40 (containing the B. subtilis secA gene) have been described previously [20]. pTRC99A, pMA12, pMKL18, and pMKL40 were transformed into MM66 and expression of the secA genes was analysed under repressed (i.e. in the presence of 0.5% glucose) and induced (i.e. in the presence of 1 mM IPTG) conditions by Western blotting using a mixture of anti-E. coli SecA and anti-B. subtilis SecA antibodies (Fig. 2A). Whereas under inducing conditions high-level synthesis of the corresponding SecA polypeptides was observed, a basal level of expression was found under repressed conditions which, most likely, is due to a leakiness of the strong trc promoter. The apparent molecular masses of the respective SecA proteins were in good agreement with the molecular masses calculated from the corresponding amino acid sequences.

Since we previously found that the growth defect of MM66 could be complemented by the B. subtilis SecA protein only under conditions of low-level synthesis [20] and since we noticed that high-level synthesis of the B. subtilis or the S. carnosus SecA protein in MM66 frequently resulted in lysis and cell
death, complementation of the growth defect of MM66 by the various SecA proteins was tested under repressed (i.e. in the presence of glucose) conditions (Table 2). Plating of pTRC99A, pMA12, pMKL18 or pMKL40-containing cells of MM66 at 42°C revealed that, in contrast to the E. coli and B. subtilis secA genes, the presence of the S. carnosus secA gene did not restore growth of MM66 at the non-permissive temperature, suggesting that the S. carnosus SecA could not functionally replace the E. coli SecA protein.

3.3. Complementation of a temperature-sensitive B. subtilis secA mutant by the S. carnosus SecA protein

To allow the functional analysis of the SecA proteins in the temperature-sensitive B. subtilis secA mutant NIG1152 (dio431') [16], the secA genes of E. coli (pWE10), B. subtilis (pWMKL1), and S. carnosus (pWA11) were cloned into the B. subtilis expression vector pWH1520 [22] under the regulatory control of the xylose-inducible xyl-promoter/operator. As shown in Fig. 2B, induction with xylose resulted in high-level synthesis of the corresponding SecA proteins in B. subtilis NIG1152. In the presence of glucose, hardly any synthesis above the level of chromosomally encoded B. subtilis SecA is detected.

The ability of the various SecA proteins to complement the growth defect of NIG1152 was tested under repressed (i.e. in the presence of glucose) and induced (i.e. in the presence of xylose) conditions (Table 3). In contrast to the E. coli secA gene, which did not restore growth of NIG1152 at the non-permissive temperature under either condition, the B. subtilis and the S. carnosus secA genes fully complemented the growth defect of NIG1152 at 42°C, regardless of the state of induction. This finding strongly suggests that even very low levels of the S. carnosus SecA protein are sufficient to substitute for the B. subtilis SecA polypeptide in growth complementation.

To test whether the S. carnosus SecA protein can in fact promote the translocation of precursor proteins in B. subtilis, pulse-chase experiments were performed monitoring the processing of a model protein (Staphylococcus hyicus pre-pro-lipase; [11]) in NIG1152 at the non-permissive temperature (Fig. 3). We have shown previously that in B. subtilis the

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**Table 2**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>secA allele</th>
<th>MM66 30°C</th>
<th>MM66 42°C</th>
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<tbody>
<tr>
<td>pTRC99A</td>
<td>None</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>pMKL18</td>
<td>E. coli</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pMKL40</td>
<td>B. subtilis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pMA12</td>
<td>S. carnosus</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Cells of E. coli MM66 carrying the respective plasmids were plated on LB plates, supplemented with 0.5% glucose, at 30°C or 42°C.

+ , growth; - , no growth.

**Table 3**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>secA allele</th>
<th>NIG1152 30°C</th>
<th>NIG1152 42°C</th>
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<tbody>
<tr>
<td>pWH1520</td>
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<td>+</td>
<td>G</td>
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<tr>
<td>pWE10</td>
<td>E. coli</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>pWMKL1</td>
<td>B. subtilis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pWA11</td>
<td>S. carnosus</td>
<td>G</td>
<td>X</td>
</tr>
</tbody>
</table>

Cells of B. subtilis NIG1152 containing the respective plasmids were plated on LB plates in the presence of 0.5% glucose (G) or 0.5% xylose (X) at 30°C or 42°C, respectively.

+ , growth; - , no growth.

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Fig. 3. Processing of pre-pro-lipase in B. subtilis secA mutant NIG1152 at 42°C. Cells of NIG1152 containing pLipPS1 [11] in addition to pWH1520 (lanes 1–4), pWMKL1 (lanes 5–8) or pWA11 (lanes 9–12) were grown in S7 medium at 42°C for 3 h, labelled with [35S]methionine for 1 min and subsequently chased with an excess of non-radioactive methionine. The samples represent chase times of 30 s (lanes 1, 5, 9), 2 min (lanes 2, 6, 10), 5 min (lanes 3, 7, 11) and 10 min (lanes 4, 8, 12). Open circle, pre-pro-lipase; arrowhead, pro-lipase; square, mature lipase-sized degradation product.
S. hyicus pre-pro-lipase is processed by signal peptidase and, after translocation across the plasma membrane, the resulting pro-lipase is further degraded to, among others, mature lipase-sized polypeptides [17]. Compared to the vector control (lanes 1-4), the presence of the S. carnosus SecA protein significantly restored processing of the pre-pro-lipase in NIG1152 at 42°C (lanes 9-12), although with lower efficiency than the B. subtilis SecA protein (lanes 5-8).

4. Discussion

In this communication, we describe the cloning and characterization of the secA gene from the Gram-positive bacterium S. carnosus. S. carnosus secA encodes a polypeptide of 844 amino acid residues which harbors significant homologies to the other known SecA proteins. Regarding the size and amino acid sequence identity, S. carnosus SecA is closely related to the SecA proteins of the Gram-positive bacteria B. subtilis (841 amino acid residues; 60.2% identity) and Listeria monocytogenes (836 amino acid residues; 60.4% identity). This is also supported by the fact that the S. carnosus SecA protein strongly cross-reacted with anti-B. subtilis SecA antibodies.

Expression of the S. carnosus secA gene in the E. coli secA mutant MM66 did not relieve the growth defect of MM66 at the non-permissive temperature under all conditions tested. In contrast and as has been reported previously [20], the B. subtilis SecA protein allowed growth of MM66 at 42°C, provided it was not overproduced. These results could reflect the fact that the B. subtilis SecA is somewhat more closely related (52.4% identity) to the E. coli SecA protein than is the S. carnosus SecA polypeptide (46.6% identity).

In contrast to its behavior in the E. coli secA mutant strain, S. carnosus SecA was fully able to complement the growth defect of the temperature-sensitive B. subtilis secA mutant NIG1152. Furthermore, the presence of S. carnosus SecA in NIG1152 resulted in substantial restoration of the secretion of a model protein (S. hyicus pre-pro-lipase) at 42°C. Although processing of pre-pro-lipase in the presence of S. carnosus SecA occurred with lower efficiency than in the presence of B. subtilis SecA, our results clearly demonstrate that S. carnosus SecA can (at least to a certain extent) functionally replace B. subtilis SecA in protein translocation. In contrast, E. coli SecA did not complement the growth defect of NIG1152 at 42°C, a finding which is consistent with the results obtained by Takamatsu et al. [16].

In summary, our results indicate that, although SecA seems to be a highly conserved component of the bacterial protein translocation apparatus, it cannot always be exchanged between different bacteria. This finding most likely reflects an optimization of the interactions between SecA and the integral components SecY/E/G of preprotein translocase and/or SecA and the corresponding precursor/chaperone complexes within each organism.

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References


