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The plasmid F OmpP protease, a homologue of OmpT, as a potential obstacle to *E. coli*-based protein production

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Abstract OmpT, an outer membrane-localized protease of *Escherichia coli*, cleaves a number of exogenous and endogenous proteins during their purification. SecY, an endogenous membrane protein, is a target of this artificial proteolysis in vitro. Here we report that SecY cleavage occurs even in cell extracts from *ompT*-disrupted cells, if they carry an F plasmid derivative. A gene, *ompP*, on the F plasmid was shown to be responsible for this proteolysis. These results indicate that the absence of an F-like plasmid should be checked when choosing a host strain for *E. coli*-based protein production.

Key words: OmpT; SecY; OmpP; F plasmid; Recombinant protein; *Escherichia coli*

1. Introduction

SecY, an integral membrane protein of *Escherichia coli* plasma membrane, is a central component of the protein translocation machinery. It forms a complex with SecE and SecG, and serves presumably as a proteinaceous channel for the passage of precursor secretory proteins (for review [1,2]). Biochemical studies of the SecYEG complex requires cell disruption and isolation of inverted inner membrane vesicles [3]. The membrane vesicles are also solubilized with a detergent for purification of the components and their reconstitution into proteoliposomes [4,5]. We reported previously that SecY is subject to cleavage in cell extracts at a central region of the protein [6]. This cleavage was due to the action of the OmpT protease present in the outer membrane. The OmpT-mediated cleavage of SecY is at a cytosolic domain and is an artificial reaction that only occurs after cell disruption. It is strikingly enhanced by detergent solubilization of a membrane preparation. Thus, in some reconstitution studies, SecY had almost totally been converted to the cleaved fragments [4,7,8]. Obviously, such an artifact should be minimized. In addition to SecY, there have been numerous cases in which either endogenous or exogenous proteins are cleaved by OmpT during their isolation [9–18]. Thus, the use of an OmpT-disrupted strain is recommendable as a host for *E. coli*-based protein production. However, disruption of the chromosomal *ompT* gene alone may not be sufficient in cases where a coexisting extrachromosomal genetic element encodes an OmpT equivalent. Here we report that F, a common episome in *E. coli*, indeed specifies a SecY-cleaving activity that can be ascribed to its *ompP* gene.

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2. Materials and methods

2.1. Bacterial strains

UT5600 was a Δ*ompT* strain [9] of *E. coli* K-12. All other strains were derivatives of strain MC4100 (F^- *araD139* Δ*(argF-lac)U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*; [19]). EM144 (MC4100 *ompT*::*kan*/F′ *lacI*^{Q} *lacPL8* $Z^+Y^+A^+$ *pro*$^+$) was constructed by introducing *ompT*::*kan* [6] into KI298 [20] by P1 transduction. EM146 and EM157 were derivatives of AD202 (MC4100 *ompT*::*kan*; [6]), into which F′ *lacI*^{Q} *lacPL8* $Z^+Y^+A^+$ *pro*$^+$ (from our stock) or F^+-1::Tn*10* (from National Institute of Genetics, Mishima, Japan), respectively, was introduced by conjugation; selection was by Lac^+ and Tet^R, respectively, while male strains were counter-selected by kanamycin. EM162 was constructed as follows. A transposon insertion, *argE*::Tn*10*, was introduced into AD202 by P1 transduction. F′112 [21] was then introduced into the transductant, selecting Arg^+ Kan^R colonies. One of them was named EM162, in which the Tn*10* insertion was lost presumably due to homogenization.

2.2. SecY cleavage in crude cell lysates

E. coli cells were grown to a mid-log phase, collected by centrifugation, washed with 10 mM Tris-HCl (pH 8.1), and resuspended in solution containing 10 mM Tris-HCl (pH 8.1), 5 mM EDTA (pH 8.0), and 5 mM β-mercaptoethanol. They were treated with lysozyme (final concentration, 1 mg/ml) on ice for 5 min followed by three cycles of freezing and thawing. After additional incubation on ice for 20 min, an equal volume of solution containing 20 mM HEPES-KOH (pH 8.0), 0.3 M KCl, 20% glycerol, and 2% Triton X-100 was added and incubation continued for another 30 min or 10 h on ice. Samples were then subjected to SDS-PAGE and immunoblotting using mixed antisera against the N-terminal and the C-terminal sequences of SecY [22]. Protein images were visualized by exposure to an X-ray film or using a LAS-1000 luminescence imager (Fuji Film).

2.3. Cloning of the ompP gene

F′ *lacI*^{Q} *lacPL8* $Z^+Y^+A^+$ *pro*$^+$ plasmid was prepared [23] from strain EM146 grown in 1 l of terrific broth. A 1.3 kb *ompP* fragment was then amplified by polymerase chain reaction using primers 5′-AACTGCAGAATGTTCAGATGACATAC-3′ and 5′-GGGGTACCAGACACAGTTCCGCAAAG-3′, which were designed to include the *Pst*I and *Kpn*I recognition sequences (underlined). The DNA fragment was digested with these enzymes, and cloned into pSTV29 [22]. The resulting plasmid pCM131 was confirmed by sequencing to contain the *ompP* coding region.

3. Results and discussion

3.1. OmpT-independent and F plasmid-dependent cleavage of SecY in vitro

F′ derivatives carrying the *lacI*^{Q} mutation are often used as a source of the overproduced *lac* repressor for controlling expression of a cloned gene under the *lac* promoter [24,25]. We prepared membrane fractions from three strains of *E. coli*, MC4100 (Δ*lac ompT*$^+$), AD202 (Δ*lac ompT*::*kan*), and EM144 (Δ*lac ompT*::*kan*/F′ *lac*$^+$ *lacI*^{Q}). They were solubilized with Triton X-100 and subjected to SDS-PAGE and SecY immunoblotting. Only AD202 (*ompT*::*kan*) gave the intact band of SecY (Fig. 1, lane 4). In contrast, no intact SecY

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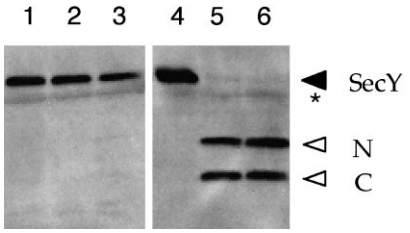

Fig. 1. In vitro cleavage of SecY in detergent solubilized membrane from an *ompT*-disrupted strain. Cells of AD202 (*ompT*::*kan*; lanes 1 and 4), EM144 (*ompT*::*kan*/F′ *lacI*^{Q} *lacPL8 Z*$^{+}$ *Y*$^{+}$ *A*$^{+}$ *pro*$^{+}$; lanes 2 and 5), and MC4100 (*ompT*$^{+}$; lanes 3 and 6), each carrying pTYE024 (irrelevant to this study; [29]), were grown at 37°C in M9-glycerol medium containing amino acids (20 μg/ml each, other than methionine and cysteine) and 20 μg/ml of chloramphenicol. Cells were disrupted by sonication, and total membranes were obtained by centrifugation [29]. Membranes were then solubilized with 1% Triton X-100 on ice for 30 min and samples were ultracentrifuged to remove insoluble materials. Samples were separated by SDS-PAGE and SecY was visualized by exposure of the immunoblot to an X-ray film. Lanes 1–3, crude cell lysate; lanes 4–6, detergent-solubilized membrane fraction. Positions of intact SecY, its cleaved N-terminal fragment, and its cleaved C-terminal fragment are shown by arrowhead on the right. Asterisk shows a non-specific background band.

band was observed for the samples from MC4100 (*ompT*$^{+}$) or EM144 (*ompT*::*kan*/F′ *lac*$^{+}$ *lacI*^{Q}). Instead, a low molecular mass band, characteristic of an OmpT-mediated cleavage product of SecY, was observed ([6]; Fig. 1, lanes 5 and 6). Such cleavage of SecY was undetectable with whole cell lysates of any strain, provided that no detergent was added and that they had been kept strictly at low temperature (Fig. 1, lanes 1–3). These results raised a possibility that the F′ *lac*$^{+}$ *lacI*^{Q} factor present in EM144 was responsible for the in vitro

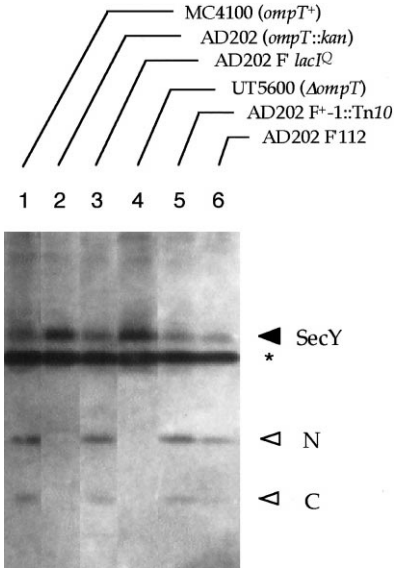

Fig. 2. F plasmid-dependent SecY cleavage. Cells of MC4100 (*ompT*$^{+}$; lane 1), AD202 (*ompT*::*kan*; lane 2), EM146 (*ompT*::*kan*/F′ *lacI*^{Q} *lacPL8 Z*$^{+}$ *Y*$^{+}$ *A*$^{+}$ *pro*$^{+}$; lane 3), UT5600 (Δ*ompT*; lane 4), EM157 (*ompT*::*kan*/F^{+}-1::Tn*10*; lane 5), and EM162 (*ompT*::*kan*/F′112; lane 6) were grown at 37°C in L medium, and cell lysates were prepared. They were treated with 1.0% Triton X-100 on ice for 30 min and subjected to SDS-PAGE. SecY was visualized by exposure of the immunoblot to an X-ray film. Complete cleavage in crude lysates required longer reaction time (data not shown).

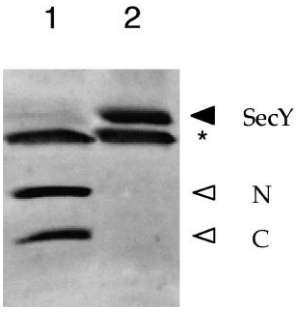

Fig. 3. OmpP-dependent SecY cleavage. Plasmid pCM131 (*ompP*; lane 1) or plasmid pSTV29 (vector; lane 2) was introduced into strain AD202. Resulting transformants were grown at 37°C in L medium containing 0.2% glucose and 10 μg/ml of chloramphenicol and cell lysates were prepared. They were treated with 1.0% Triton X-100 on ice for 10 h, and subjected to SDS-PAGE. The SecY immunoblot was visualized using a LAS-1000 luminescence imager.

cleavage of SecY in the detergent solubilized membrane preparation from this *ompT*-disrupted strain.

We introduced three different derivatives of the F factor, F′ *lac*$^{+}$ *lacI*^{Q}, F^{+}-1::Tn*10*, or F′112, into AD202, and cell lysates were prepared in the presence of Triton X-100. As shown in Fig. 2 (lanes 3, 5, and 6), cells carrying any one of these derivatives produced the cleaved fragments of SecY, whereas *ompT*-disrupted strains without an F factor contained exclusively the intact SecY protein (Fig. 2, lanes 2 and 4). F^{+}-1::Tn*10* carried no chromosomal gene, whereas F′ *lac*$^{+}$ *lacI*^{Q} and F′112 carried different chromosomal segments. These observations suggested that the F plasmid itself, rather than a 'cloned' chromosomal gene, was responsible for the SecY-cleaving activity.

3.2. OmpP encoded by the F plasmid is responsible for the SecY cleavage

Kaufmann et al. [26] described a gene *ompP*, which encodes the precursor form of an outer membrane protein with sequence identity of about 70% with OmpT. OmpP exhibits OmpT-like proteolytic activities. Although this gene was detected in certain subsets of *E. coli* strains, its exact origin remained elusive. On the other hand, Shimizu et al. [Shimizu, H., Uehara, K., Sampei, G. and Mizobuchi, K., Abstract in the Annual Meeting of the Japanese Society for Mol. Biol. (1998) p. 232] recently determined the entire nucleotide sequence of the F plasmid. Interestingly, F was found to possess a segment of 2269 bp whose sequence was exactly the same as that reported by Kaufmann et al. [26]. Thus, the F plasmid encodes the ompT-like protein.

To address whether the F plasmids we used indeed encoded the *ompP* gene, we carried out polymerase chain reactions using template DNAs from the strains used in Fig. 2 and primers flanking the F factor *ompP* gene. A fragment of 1.3 kb was amplified in common from the strains that were SecY cleavage positive but *ompT* negative (data not shown). This fragment was cloned into vector pSTV29. The resulting plasmid pCM131 indeed proved to contain a segment of the identical sequence as *ompP*. This plasmid was introduced into AD202 to examine whether it conferred a SecY cleavage activity to cell lysates in the presence of Triton X-100. As shown in Fig. 3, the cells carrying pCM131 gave the two cleaved fragments of SecY, whereas those carrying the vector alone gave exclusively the intact SecY protein. Since there was com-

plete correlation among the presence of SecY cleavage activity, the presence of an F plasmid derivative, and amplification of the 1.3 kb DNA fragment, we concluded that F-encoded OmpP protein acts like the OmpT protease in artificially cleaving the SecY protein. Thus, we believe that the *ompP* gene, reported originally by Kaufmann et al. [26], is actually present on either an autonomous or an integrated state of the F factor.

3.3. Choosing host cells for protein production in biotechnology and research

It is well conceivable that not only SecY but also the other OmpT-susceptible proteins are the proteolytic targets of this F-encoded protease. The physiological roles of OmpT have been speculated to be in certain aspects of pathogenicity of Gram-negative bacteria as well as their cell surface organization (for a review [27]). It may also confer bacterial resistance to certain antimicrobial peptides [28]. The existence of the *ompT* homologue in the F plasmid suggests that this class of cell surface proteases give some advantage in survival or transmission of the plasmid.

Whatever the intrinsic roles of OmpP might be, our present results call for attention in using particular *E. coli* strains for biotechnological and research purposes. OmpT-deficient strains have been recommended as a host for production of recombinant proteins, including GST fusion proteins [16], as well as native *E. coli* proteins, including SecY [6]. There are numerous examples of OmpT-mediated cleavage of proteins during their isolation [6,9–18]. To circumvent such unwanted proteolysis, the absence of F-like plasmids should also be taken into account. Some of the commonly used strains for recombinant DNA work indeed carry an F plasmid derivative [23–25].

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