Expression of the pCloDF13 encoded bacteriocin release protein or its stable signal peptide causes early effects on protein biosynthesis and Mg\textsuperscript{2+} transport

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Abstract

The effect of the pCloDF13 encoded bacteriocin release protein (BRP) on Escherichia coli cell lethality was studied. Induction of the BRP resulted in a strong inhibition of the incorporation of radioactive labeled amino acids and affected the transport of Mg\textsuperscript{2+} ions. Similar effects were obtained when the BRP stable signal peptide was expressed as a separate entity. Kinetic studies revealed that these effects occurred prior to quasi-lysis and release of cloacin DF13. The results indicated that the BRP induced cell lethality is caused by early effects on protein synthesis and Mg\textsuperscript{2+} transport, due to the accumulation of stable BRP signal peptides in the cytoplasmic membrane.

Bacteriocin release proteins (BRPs, also called lysis proteins or kil proteins) are involved in the semi-specific transfer of bacteriocins, like colicins and cloacin DF13, across the cell envelope of Escherichia coli. Expression of BRPs also results in a decline in culture turbidity (called quasi-lysis or ‘lysis’), an inhibition of colony formation on agar plates (lethality), and in a leakage of periplasmic proteins from the host cells (Pugsley 1984; De Graaf & Oudega 1986). The effects of the pCloDF13-derived BRP on quasi-lysis and release of cloacin DF13 have been studied extensively. The pCloDF13-derived BRP is synthesized as a precursor lipoprotein of 49 amino acid residues. The preBRP is slowly processed by the lipoprotein specific signal peptidase II (SPaseII), rendering a lipid-modified mature BRP which is primarily located in the outer membrane of E. coli (Oudega et al. 1984; Van der Wal et al. 1992). In the outer membrane, the so-called detergent-resistant phospholipase A is activated. This activation is essential for quasi-lysis and release of cloacin DF13 or periplasmic proteins (Luirink et al. 1986). Divalent cations, like Mg\textsuperscript{2+} and/or Ca\textsuperscript{2+} in the culture medium of cells expressing the BRP interfere with the BRP-dependent quasi-lysis, but hardly affect protein release (Luirink et al. 1986). The signal peptides of most BRPs are unusual in that they are not proteolytically degraded after processing of the preBRP (Cavard et al. 1987; Pugsley & Cole 1987; Luirink et al. 1989). The stable signal peptides accumulate in the cytoplasmic membrane (Howard et al. 1991; Van der Wal et al. 1992). The role of the pCloDF13-derived stable BRP signal peptide has been investigated by genetically exchanging the stable BRP signal peptide for the unstable Lpp signal peptide. The resulting hybrid BRP, correctly targeted by the Lpp signal peptide (Gennity et al. 1992; Van der Wal et al. 1992), did not function in the release of cloacin DF13, but its expression did result in quasi-lysis, lethality and leakage of periplasmic proteins (Luirink et al. 1991). The BRP signal peptide expressed alone is not able to provoke the release of cloacin DF13, but causes quasi-lysis and lethality (Van der Wal et al. 1992). So far, not much information is available on the mechanism of lethality caused by the pCloDF13-encoded BRP or derivative polypeptides. Relatively low expression of this BRP already results in an inhibition of colony formation on broth agar plates, but also in a release of cloacin DF13 (Luirink et al. 1987). In liquid media, a
relatively low expression of the BRP does not result in a decline in culture turbidity or in a degradation of peptidoglycan (laurink et al. 1986). These effects can only be observed after relatively high expression of the BRP. The first effect found upon high expression of the BRP is a decline in culture turbidity, which probably results from alterations in the bacterial membranes. Concomitant with this decline, a release of cloacin DF13 can be observed and finally, at a much later stage, a degradation of peptidoglycan (cell lysis) can be detected (Luirink et al. 1986; Cavard & Oudega 1992; Luirink et al. 1992). We are interested in the mechanism of lethality caused by the expression of the pCloDF13-encoded BRP and studied the effect of the BRP on the functioning of the cytoplasmic membrane. One of our hypotheses was that the accumulation of BRP precursors and/or cleaved but stable BRP signal peptides in the cytoplasmic membrane, might affect the processing of precursors of periplasmic or outer membrane proteins. This 'traffic jam' might be the cause of the observed cell lethality. To study this possibility, cells of\textit{E. coli FTP4170} \(\Delta(\text{tonB trpAE})\ argE(Am)\) glyV55 (Luirink et al. 1989) were transformed with plasmid pJL17. This plasmid is a derivative of pINIIIA1 (Masui et al. 1984) containing the wild-type gene encoding the pCloDF13-encoded BRP downstream of the isopropyl-\(\beta\)-D-thio-galacto-pyranoside (IPTG)-inducible \(lph/lac\) promoter/operator (see Fig. 1; Luirink et al. 1988).

These cells were pulse-labeled with \([35S]\)methionine at various time points after induction of the BRP (results not shown). However, the incorporation of radio-labeled methionine into these proteins was affected within one hour after induction started. In control cells, not induced with IPTG, or in cells harboring the plasmid vector pINIIIA1 (not coding for BRP), this phenomenon was not observed. Therefore, it appeared that expression of the BRP affected the incorporation of labeled methionine into these proteins.

To further study the effect of BRP expression, cells were induced with IPTG and pulse-labeled with \([35S]\)methionine at 30 min intervals. The incorporation of label into cellular proteins was then analyzed by SDS-PAGE (Fig. 2). Induction of the BRP apparently affected the biosynthesis of all proteins within one hour (see lanes 2 and 6). At 90 and 120 min after induction, the biosynthesis of radio-labeled proteins appeared to be almost completely blocked (lanes 10 and 14). In control experiments, no inhibition of the incorporation of labeled methionine was observed (see other lanes in Fig. 2).

These results suggested a strong effect of BRP expression on total protein synthesis. A more kinetic analysis was carried out by measuring simultaneously the decline of culture turbidity after BRP induction and the incorporation of \(^3\)H-labeled amino acid residues into trichloroacetic acid (TCA) precipitable proteins (Fig. 3). Following induction of the BRP, incorporation of \(^3\)H-amino acids was clearly hampered \(30\) min after induction, whereas a decline in culture turbidity was only detectable after 50 min. Comparable results were also obtained when plasmid pJL38 was used. This plasmid is a derivative of pJL17 carrying a stopcodon after the part of the BRP gene encoding the stable signal sequence (see Fig. 1; Van der Wal et al. 1994). Induction of this plasmid results in the expression of only the stable BRP signal peptide, which accumulates in the cytoplasmic membrane (Van der Wal et al. 1992). This indicated that not only expression of the BRP results in an inhibition of protein synthesis but also the expression of the stable signal peptide alone. Finally, also cells of \textit{E. coli FTP4170} harboring pJN73 were used...