Bacteriocin Release Protein Triggers Dimerization of Outer Membrane Phospholipase A In Vivo

NIEK DEKKER,†* JAN TOMMASSEN,‡ AND HUBERTUS M. VERHEIJ†‡

Department of Enzymology and Protein Engineering, Center for Biomembranes and Lipid Enzymology,§ and Department of Molecular Cell Biology,¶ Institute of Biomembranes, Utrecht University, 3584 CH Utrecht, The Netherlands

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Bacteriocin release protein is known to activate outer membrane phospholipase A (OMPLA), which results in the release of colicin from Escherichia coli. In vivo chemical cross-linking experiments revealed that the activation coincides with dimerization of OMPLA. Permeabilization of the cell envelope and dimerization were characterized by a lag time of 2 h.

Colicins are plasmid-encoded proteins produced by Escherichia coli and capable of killing E. coli and closely related species (18). Bacteriocin release protein (BRP) (also referred to as lysis protein) is a small lipoprotein that is essential for the release of colicin into the medium. BRP activates the endogenous outer membrane (OM) phospholipase (OMPLA) (6, 14, 20) which is important for the permeabilization of the cell envelope, since strains defective in the structural gene for OMPLA, pldA, do not release colicin. OMPLA is constitutively expressed, but enzymatic activity is detected in vivo only under adverse conditions (2, 7, 15, 17). Chemical cross-linking with formaldehyde suggested that OMPLA is present in whole cells in a monomeric state, whereas the dimeric form of the enzyme could be detected after sonication of the cells (10). We have shown in vitro that the monomeric state of OMPLA is inactive and that activation requires dimerization (10). In this study, we have investigated whether under physiologically relevant conditions activation of OMPLA involves dimerization.

The pACYC184-based plasmid pJL4 encodes chloramphenicol acetyltransferase (CAT) and contains the BRP gene from pCloDF13 of Enterobacter cloacae under control of the lpp/lac tandem promoter/operator (13). Plasmid pRB1 (4) contains the wild-type pldA gene under its own, constitutive promoter; pRB1-S144A (5) contains a pldA allele, which encodes an inactive S144A substitution mutant OMPLA. A 3,300-bp XmnI/NruI fragment of plasmid pMF20 (22), carrying the phoA gene encoding alkaline phosphatase (AP) under control of a constitutive, mutant promoter was subcloned into the unique HindII site of pRB1 and of pRB1-S144A, yielding plasmids pND19 and pND20, respectively. E. coli K-12 strain CE1303 (pldA recA56 [9]) transformed with pJL4 and either pND19 or pND20 was grown under agitation at 37°C in Luria broth supplemented with chloramphenicol (37 µg/ml) and ampicillin (50 µg/ml) and with 10 mM MgCl₂ to prevent BRP-induced lysis (14, 20). When the optical density at 600 nm reached 0.3, 150 µM isopropyl-ß-D-thiogalactopyranoside (IPTG) was added to induce BRP synthesis.

The permeabilization of the cell envelope was monitored by measuring the release of cytosolic CAT and of periplasmic AP into the medium. Measurement of CAT activity (23) revealed the presence of 1 to 2% of the total CAT activity in the medium before induction of BRP synthesis (Fig. 1). After induction, CAT activity in the medium increased to 10% of the total amount after a lag period of approximately 2 h (Fig. 1). This lag time is consistent with previous observations for the release of colicin E2 (20), cloacin DF13 (14) and marker enzymes (19). The release of CAT was dependent on the action of OMPLA, since it was much lower when an inactive mutant of OMPLA, in which the active-site serine was replaced by an alanine, was used (Fig. 1). However, also in the latter case, the levels of CAT in the supernatant were significantly higher than when BRP synthesis was not induced (Fig. 1), indicating that BRP alone is capable of a partial permeabilization of the cell envelope. Measurement of AP activity (26) revealed the presence of 5% of the total amount of AP produced in the medium when BRP synthesis was not induced. After induction of BRP synthesis, the release of AP increased to 27% of the total activity (data not shown). The release of AP was dependent on the activity of wild-type OMPLA and was again characterized by a lag time of 2 h (data not shown). These data show that periplasmic AP can enter the secretion pathway, suggestive of a sequential secretion process.

Dimerization of OMPLA was analyzed in vivo by chemical cross-linking with formaldehyde as described previously (10). Without induction of BRP synthesis, OMPLA was detected mostly as a monomeric species (Fig. 2, odd-numbered lanes). However, when BRP synthesis was induced, considerable amounts of dimer could be trapped (Fig. 2, lanes 4, 6, 8 and 10). The absence of dimeric cross-linking products before activation could be explained by the existence of OMPLA in a dimeric but cross-linking-incompetent state under normal conditions. However, when glutaraldehyde was used as a cross-linker with a longer spacer, dimers were also not observed (results not shown). Therefore, it is unlikely that OMPLA preexists in a dimeric state in the OM, although this possibility cannot be totally excluded. The dimerization of OMPLA was characterized by a considerable lag time, which correlates with the lag time observed for the release of CAT and AP into the medium. Moreover, it has been reported that the formation of lysophosphatidylethanolamine, the major hydrolization product of the OMPLA-catalyzed reaction, is characterized by a similar lag time observed for the release of CAT and AP into the medium (results not shown). Therefore, it is unlikely that OMPLA preexists in a dimeric state in the OM, although this possibility cannot be totally excluded. The dimerization of OMPLA was characterized by a considerable lag time, which correlates with the lag time observed for the release of CAT and AP into the medium. Moreover, it has been reported that the formation of lysophosphatidylethanolamine, the major hydrolization product of the OMPLA-catalyzed reaction, is characterized by a similar lag time (6, 14, 20). BRP-induced dimerization was also detected for the inactive mutant OMPLA (data not shown). What can we propose now for the molecular mechanism of BRP-mediated activation of OMPLA? The lag time of 2 h...
after induction of BRP expression before the onset of permeabilization of the cell envelope and dimerization of OMPLA is intriguing. Although the posttranslational processing of BRP is a slow process and takes minutes for completion (11), it is still rapid compared to the observed lag time. BRP is produced in large amounts up to $10^5$ copies per cell (12), which are much larger than the small amounts of OMPLA present. Therefore, a specific, direct interaction between BRP and OMPLA is not very likely the direct trigger for activation. Diffusion rates within the OM are very low (25) due to the interactions between the negatively charged lipopolysaccharide molecules, which are inter interconnected via Mg$^{2+}$ or Ca$^{2+}$-mediated salt bridges and via hydrophobic interactions between the lipid A parts (16). The low diffusion rate together with the low abundance of the protein (380 copies per cell [21]) might normally prevent the dimerization of OMPLA. The incorporation of BRP in the OM could destabilize the membrane, especially if the localization is restricted to one leaflet (24). Such action correlates with our finding that, although with reduced efficiency, BRP alone is capable of permeating the OM. Morphological changes induced by BRP have been observed as blebs in the OM (27). Furthermore, low-level expression of the pColE1 BRP causes partial exfoliation of the OM (1). One can envisage that the BRP-mediated membrane perturbation relieves the lipid asymmetry of the OM and accelerates diffusion in the OM, thereby triggering OMPLA activation. In our model, we assume a uniform distribution of monomeric OMPLA within the OM. Recently, a method for the specific in vivo labeling of cytochrome residues in the OM protein FluH has been reported (3). The labeling of single-cytochrome mutants of OMPLA with fluorescent dyes could provide insight in the distribution of OMPLA over the cell surface, and we are currently investigating this possibility. Once activated, the enzyme will generate fatty acid and, more importantly, lysophospholipid, a compound known to destabilize membranes (8). The water solubility of lysophospholipid is likely sufficient to permit transport to the cytoplasmic membrane. The presence of large amounts of BRP and lysophospholipid in both membranes could allow for the direct transport of colicins and reporter enzymes through these membranes in a sequential manner.

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REFERENCES


