Phosphatidylethanolamine mediates insertion of the catalytic domain of leader peptidase in membranes

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Abstract **Leader peptidase is an integral membrane protein of *E. coli* and it catalyses the removal of most signal peptides from translocated precursor proteins. In this study it is shown that when the transmembrane anchors are removed in vivo, the remaining catalytic domain can bind to inner and outer membranes of *E. coli*. Furthermore, the purified catalytic domain binds to inner membrane vesicles and vesicles composed of purified inner membrane lipids with comparable efficiency. It is shown that the interaction is caused by penetration of a part of the catalytic domain between the lipids. Penetration is mediated by phosphatidylethanolamine, the most abundant lipid in *E. coli*, and does not seem to depend on electrostatic interactions. A hydrophobic segment around the catalytically important residue serine 90 is required for the interaction with membranes.**
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Key words: Leader peptidase; Membrane protein; Protein-lipid interaction; Protein secretion; Phosphatidylethanolamine; Insertion

1. Introduction

For recognition by the export machinery, proteins are usually synthesized as precursors with amino terminal extensions called leader or signal sequences. Signal sequences contain a hydrophobic core region which is preceded by an amino terminal positively charged domain [1]. After translocation signal sequences are removed by the action of the

56[(seque54duction4al)]TJIhggvec2M88-380[(se14egion)-39quen5(0vby)-15(5^7y)-15(n35du0f(se1h
-360(u19.1843J -0.9991 -1.22ic)-430((are)-38550

bated at room temperature for 10 min. Cells were pelleted and the pellet was quickly resuspended in ice cold 0.5 mM $MgCl_2$ and incubated on ice for 10 min. After centrifugation for 8 min the supernatant was separated from the pellet. Both pellet and supernatant fractions were immunoprecipitated with Lep and β-lactamase antibodies and analysed by SDS-PAGE followed by autoradiography.

Inner and outer membrane fractions of MC1061 expressing H2-CC were essentially prepared as described in the next section. Cells were grown in LB medium supplemented with ampicillin (50 μg/ml) and induced with 0.2% arabinose in early exponential phase. After one hour of induction the cells were harvested by 15 min centrifugation at 6 krpm in a GSA rotor (Sorvall).

2.3. Vesicle isolation and preparation

Inverted and right-side out inner membrane vesicles and outer membranes of E. coli strain MC1061 were isolated according to published procedures [16,17]. The identity of the fractions was confirmed by lipopolysaccharide staining and immunodetection of OmpA, Lep and SecY. Large unilamellar vesicles (LUVETs) were prepared by means of extrusion through a polycarbonate filter (Nucleopore; 0.2 μm pore size) of a rehydrated (10 mM Tris-HCl, 50 mM NaCl, pH 7.5) total lipid extract from the inner membrane of MC1061.

2.4. Vesicle binding assay

LUVETs corresponding to 200 nmol lipid were incubated with the indicated amounts of protein (Fig. 3B) in 300 μl 10 mM Tris-HCl, 50 mM NaCl, pH 7.5, for 1 h at room temperature. Inner membrane vesicles and outer membranes were incubated in 300 μl 50 mM triethanolamine/HAc, 250 mM sucrose, 1 mM DTT, pH 7.5, to maintain the same environment as during isolation. Vesicles were pelleted by centrifugation for 30 min at 236×10^3 g at room temperature in a TLA 100.3 rotor using a TL 100 ultracentrifuge (Beckmann Instruments Inc., Palo Alto, CA, USA). Pelleting efficiencies of the vesicles were calculated after phosphorus determination [18] on supernatant and pellet. The amount of bound protein was determined after SDS-PAGE and Coomassie Brilliant Blue staining of pellet and supernatant fractions. The intensities of the bands were quantified by densitometry (Personal Densitometer, Molecular Dynamics, Sunnyvale, CA, USA) and compared to calibration curves of the same proteins which were run on the same gels. The amount of bound protein was corrected for the pelleting efficiency of the vesicles which was always

imately 30 kDa, which is the size expected for the cleaved P2 domain, was associated with both membranes with a slight preference for the outer membrane (Fig. 1B, squares).

The ability of the periplasmic domain of Lep to bind to membranes was confirmed by vesicle binding experiments. For this purpose we made use of a purified, enzymatically active construct (Δ2-75, see Fig. 3A) lacking the H1-P1-H2 domain [9]. By means of ultracentrifugation experiments the binding of Δ2-75 to right-side-out inner membrane vesicles and outer membranes was determined. In the absence of membranes, Δ2-75 was quantitatively recovered in the supernatant after ultracentrifugation (Fig. 2, upper panel, lanes 1–3). Right-side out inner membrane vesicles (Fig. 2, upper panel, lanes 4–6) contain many different proteins as judged by Coomassie Brilliant Blue staining of gels, while outer membranes (Fig. 2, lower panel, lanes 1–3) show a characteristic pattern with only two dominant bands. Both types of vesicles were pelleted efficiently. When Δ2-75 was incubated with vesicles prior to centrifugation, a significant fraction of the molecules sedimented with the vesicles (Fig. 2, upper panel, lanes 7–9 and lower panel, lanes 4–6). Thus, Δ2-75 is apparently capable of binding to both inner and outer membranes while the native population of Lep is found mostly in the inner membrane. This suggests that the periplasmic domain does not require specific inner membrane components for binding. To investigate the possibility that the P2 domain recognises the lipid component of membranes, the binding of 5 μg Δ2-75 to inner membranes and to unilamellar lipid vesicles made from purified inner membrane lipids were compared. The same amount of lipid phosphorus was used for both types of vesicles. Right side out inner membrane vesicles bound 1.7 ± 0.3 μg of Δ2-75 while the lipid vesicles bound 1.6 ± 0.3 μg, implying that membrane binding of Δ2-75 is m2-7edicentrif[9]ni-Righttoftisight

tial surface pressure at which a protein can cause an increase in surface pressure. This parameter is a measure of the membrane-penetrating ability of the protein. In biological membranes the packing densities of the lipids correspond to surface pressures between 31 and 35 mN/m [23]. To investigate

lipid extract the PE component is responsible for insertion of Δ2-75. The preference of the periplasmic domain for binding to the outer membrane (Fig. 1B) is compatible with a speci¢c binding to the PE component, since the periplasmic lea£et of the outer membrane is enriched in PE over the inner membrane [28].

The zwitterionic nature of the PE headgroup is not responsible for the speci¢c interaction with the catalytic domain of leader peptidase because insertion into monolayers of the zwitterionic PC is greatly reduced. In recent years it has become increasingly clear that PE has special properties allowing it to mediate membrane insertion and binding of proteins (for review see [29]). Examples include insertion of the chloroplast precursor protein of ferredoxin [21], the precursor of the E. coli pore protein PhoE [30], SecA [27], and blood coagulation factor VIII [31]. Furthermore, PE promotes folding of a periplasmic loop of newly inserted lactose permease [32], it regulates the activity of glycerophosphate acyltransferase [33], it is essential for e⁄ cient functioning of preprotein translocase [34], and was recently found in crystals of cytochrome c oxidase [35].

The hypothesis has been put forward [36] that PE as non-bilayer lipid with its small headgroup in conjunction with a strong tendency of this lipid to organize in structures with a high intrinsic negative surface curvature when constrained within a bilayer, lowers the lateral pressure in the interface. This could create insertion sites for proteins or for other amphipatic components as the anti-cancer drug doxorubicine [37]. It is this property that may be responsible for insertion of the catalytic domain of leader peptidase into the periplasmic lea£et of the E. coli inner membrane.

It is not possible to draw ¢rm conclusions about the part of P2 which is responsible for the interactions with membranes containing phosphatidylethanolamine. However, considering the nature and speci¢city of the interaction between the periplasmic domain of Lep and lipids it is most likely that membrane association is caused by interaction of a hydrophobic segment within the periplasmic domain with the lipids. Deletion of H3 (residues 83^98) which is the most hydrophobic part within the periplasmic domain indeed diminished both insertion into the lipid monolayers and association with the lipid vesicles.

The insertion into the lipid phase as described in this study has important implications for the mode of action of Lep. Because of the short hydrophobic core and the cytosolic localisation of the N-termini of signal sequences, the catalytic site of Lep must be very close to the membrane. Insertion of the periplasmic domain into the lipid phase and the possible involvement of H3, which carries the catalytic serine-90 residue, implies that the active site of Lep may be at least partially buried in the membrane.

Interestingly, it has been observed that lengthening the hydrophobic core of signal sequences results in reduced processing of preproteins without much e¡ ect on translocation [38]. Our data suggest that the signal peptide cleavage site may have been moved too far out of the membrane to be accessible to the active site of leader peptidase under these conditions.

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