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PREDICTION OF SIGNAL SEQUENCE-DEPENDENT PROTEIN TRANSLOCATION IN BACTERIA: ASSESSMENT OF THE ESCHERICHIA COLI MINICELL SYSTEM

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The use of phenethyl alcohol (PEA) as a probe for signal sequence-dependent protein translocation in minicells was examined. Processing of β-lactamases and tonA was inhibited by PEA at concentrations which did not affect production of the α and γ forms of penicillin binding protein (PBP) 1b. The PBP1bs are believed to lack leader sequences whereas the other proteins contain them. Processing of a β-lactamase which shares the murein-lipoprotein export pathway was relatively resistant to PEA, consistent with previous findings in whole bacteria. The results reported here suggest that PEA is a suitable probe for leader sequences in the minicell system. By using PEA we predict that PBP4 does not require a leader sequence for membrane insertion.

During bacterial growth many proteins are inserted into the cell envelope. In E. coli most periplasmic and outer membrane proteins and some cytoplasmic membrane proteins, are synthesised as precursors containing an amino-terminal extension known as the signal sequence or leader peptide, which facilitates protein translocation through the cell envelope (1,2). The signal sequence is removed by leader peptidase activity before protein maturation (1,2). In contrast, some envelope proteins, particularly those associated with the cytoplasmic membrane, are inserted by a process that does not depend upon possession of a leader sequence (1,2). In the latter case these inner membrane proteins may simply partition into the membrane after synthesis is complete (1). The ability to detect, or predict, the presence of a leader sequence during synthesis of an envelope protein is therefore helpful when formulating models for membrane protein assembly.

A number of approaches have been used to detect, or predict, signal sequences in proteins (1,2). The simplest predictive method proposed, especially for the products of plasmid-located or cloned genes, involves translation in E. coli minicells under conditions where processing is thought to be specifically inhibited by low molecular weight alcohols. In this system a putative precursor protein (preprotein) is detected as a higher molecular weight form of the mature product (3-5). The reliability of alcohols as probes for leader sequences in minicells has not been fully evaluated, but

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depends upon the assumption that insertion of proteins into minicell and whole cell envelopes is comparable. However, this assertion may be untrue because minicell and whole cell envelopes differ structurally (6,7), expression of some transport proteins is impaired in minicells (8) and proteins located in the outer membrane of whole cells may fail to be exported in minicells (9). Phenethyl alcohol (PEA), which inhibits processing of envelope proteins in whole E. coli, has been suggested as a particularly useful probe for leader sequences (10). However, its effects on the synthesis of proteins in minicells has not been studied. In view of this we have examined the effects of PEA on the production, in minicells, of membrane proteins known either to be synthesized as pre-proteins, or which lack leader sequences.

MATERIALS AND METHODS

Bacterial strains: The E. coli minicell producing strain DS410 (11) was transformed (11) with plasmids pUC9 (12), pRW83 (13), pLC19-19 and pLC18-38 (14) to produce independent DS410 derivatives each carrying a separate plasmid.

Purification of minicells, labelling of proteins, gel electrophoresis and detection of labelled proteins: These procedures were performed as described (11).

Quantification of peak areas: This was performed by scanning densitometry of flurograms with a Joyce-Loebl Chromoscan 3 (15).

RESULTS

PEA inhibits processing of envelope proteins in whole E. coli cells (10), but its effect on synthesis of proteins in minicells has not been examined. Plasmid pUC9 encodes a TEM1 β-lactamase which is known to be synthesised as a pre-protein (1). Precursor and processed forms of this enzyme were observed in minicells (Fig. 1, bands 1' and 1

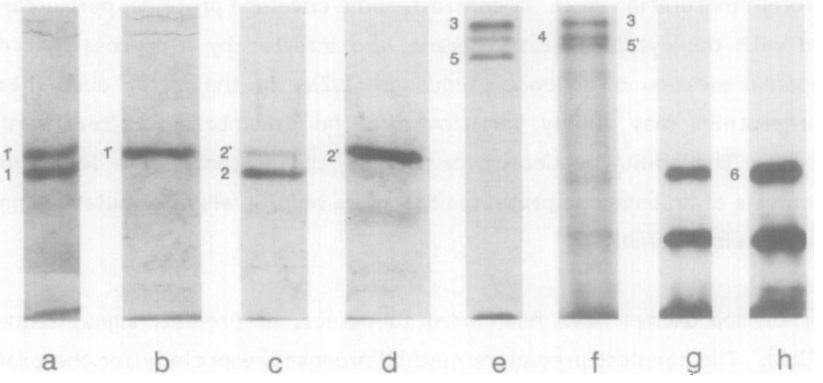

Figure 1.

Flurogram of [^{35}S]-labelled proteins synthesized in minicells containing plasmids pUC9 (tracks a,b), pRW83 (tracks c,d) pLC19-19 (tracks e,f) and pLC18-38 (g,h). The profiles in tracks a,c,e,g were obtained after labelling in the absence of PEA. The other profiles were obtained from minicells exposed to PEA during protein labelling: tracks b,f,h 0.2% (v/v) PEA; track d 0.35% (v/v) PEA. The numbers show the position of the processed and precursor forms of the pUC9 encoded β-lactamase (1,1' respectively), the processed and precursor forms of the pRW83 encoded β-lactamase (2,2' respectively), the α and γ forms of PBP1b (3,4, respectively), the processed and precursor forms of tonA (5,5' respectively), and PBP4 (6).

respectively), consistent with previous reports that processing of this enzyme is incomplete in minicells (1,7). Addition of PEA during synthesis of TEM1 led to complete inhibition of processing (Fig. 1, track b). Plasmid pRW83 encodes a β-lactamase whose synthesis and secretion closely resembles that of murein lipoprotein (16,17). As with pUC9, precursor and processed forms of the β-lactamase were visualised (Fig. 1, track c, bands 2' and 2), and processing was inhibited in the presence of PEA (Fig. 1, track d). Processing of murein lipoprotein is relatively resistant to inhibition by PEA (10) and this was also demonstrated for the pRW83 encoded enzyme since higher concentrations of PEA were required to inhibit processing of the pRW83 enzyme when compared to TEM1 processing (Fig. 2).

Plasmid pLC 19-19 carries the genes tonA and ponB which encode respectively the tonA protein and penicillin binding protein (PBP) 1b, the latter synthesized in minicells in two forms, PBP1bα and PBP1bγ (18,19). These PBPs are not synthesized as pre-proteins whereas tonA is (18,19). Minicells carrying pLC 19-19 expressed three high molecular weight polypeptides corresponding to PBP1bα, PBP1bγ and tonA (Fig. 1, track e, bands 3,4,5). Exposure to PEA led to synthesis of a higher molecular weight form of tonA whereas synthesis of the PBPs was unaffected by exposure to the alcohol (Fig. 1, track h).

Plasmid pLC 18-38 encodes PBP4 (14). It is not known whether PBP4 is synthesised as a precursor, but addition of PEA during synthesis did not result in the formation of a larger molecular form of the protein (Fig. 1, tracks g,h).

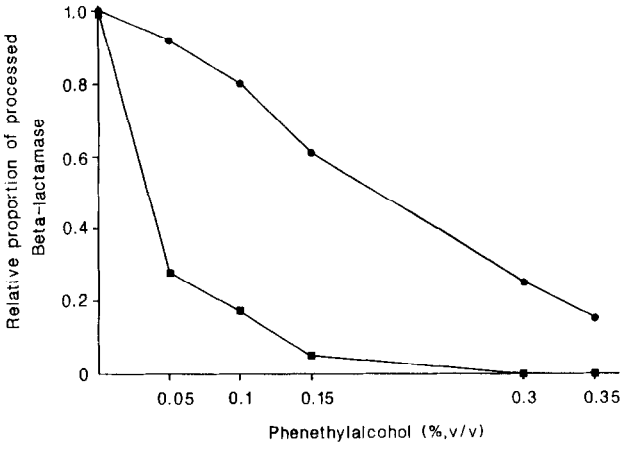

Figure 2.

Susceptibility of β-lactamase processing in minicells to PEA. PEA at the concentrations indicated was added during labelling of proteins in minicells carrying pRW83 (●) or pUC9 (■). Labelled products were separated by gel electrophoresis and detected by flurography. The bands corresponding to the processed forms of each β-lactamase were quantified by scanning densitometry and the amounts synthesised expressed as a proportion of the processed forms present in the PEA-free controls.

DISCUSSION

Reservations concerning the use of alcohols in minicells as probes for leader sequences have been raised by others (3). However, our results suggest that PEA is an appropriate probe in the minicell system because it (a) inhibits the processing of proteins known, or believed to be synthesised as precursors, and (b) its presence during synthesis of proteins lacking leader sequences does not affect their electrophoretic mobility. Furthermore, we have also demonstrated that processing of the pRW83 β-lactamase which shares the murein-lipoprotein export pathway (16,17) is relatively resistant to inhibition by PEA, consistent with the reported resistance to PEA of murein lipoprotein processing in whole bacteria (10). From results presented here (Fig. 1) we predict that PBP4 is not synthesised as a precursor. Since PBP4 is a cytoplasmic membrane protein (20) its insertion into the cell envelope may not require a leader sequence. Confirmation that PBP4 lacks a leader peptide awaits analysis of nucleotide sequencing data.

The exact mechanism by which PEA inhibits protein translocation is unknown, but it may distort membrane conformation to the point where precursor proteins are prevented from interacting effectively with leader peptidases, also located in the cytoplasmic membrane (21). Our data suggest that PEA has similar effects in whole cells and minicells.

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