

Signal peptide cleavage in the *E. coli* membrane

The 2001 Merck Frosst Prize Award Address

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Abstract

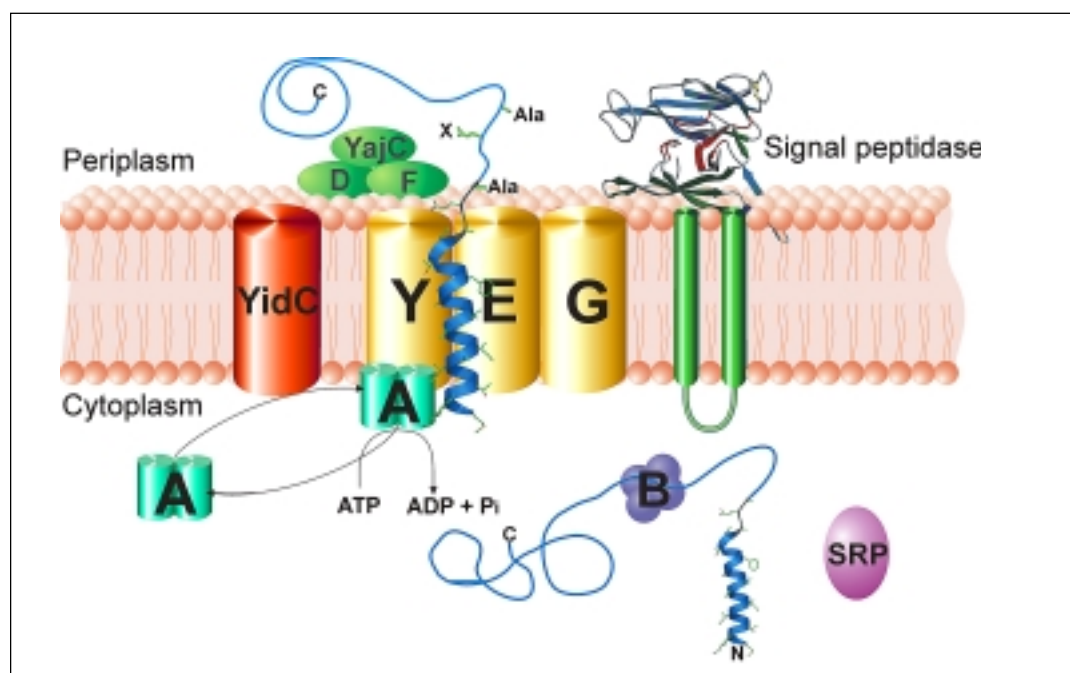
Our laboratory uses x-ray crystallography and other structural biology techniques in the analysis of bacterial membrane proteins and membrane protein complexes. We are particularly interested in bacterial protein secretion machinery, including the Sec-dependent translocation apparatus which is essential to the viability of all bacteria, as well as the type III secretion apparatus which is specific to bacterial pathogens. The latter is a fascinating system which apparently acts as a “molecular syringe” to inject virulence proteins directly from the pathogenic bacteria into the host cell. In this paper we focus on one step of the Sec-dependent translocation of proteins, the essential cleavage

of the signal peptide from membrane embedded pre-proteins during the translocation cycle.

Introduction

Many of the proteins that are essential for the survival of the bacterial cell function on the outside (or trans side) of the bacterial inner membrane. For example, proteases and beta-lactamases involved in defense mechanisms and transpeptidases involved in the synthesis of the cell wall must first be exported across the lipid bilayer of the inner membrane.

The majority of the proteins that are destined for translocation across the bacterial inner membrane are synthesized as pre-proteins with an amino-terminal peptide extension called the signal



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Figure 1. A schematic diagram of the Sec dependent protein translocation system. The pre-protein with N-terminal signal peptide interacts with the homo-tetramer SecB which chaperones the protein to the homo-dimer SecA which works as a molecular motor to push the pre-protein through a pore formed by SecYEG. The proteins SecDF and YajC function to refold the protein on the trans side of the membrane. Once translocated across the membrane the signal peptide is cleaved off by the type I signal peptidase.

or leader peptide. The signal peptide is essential for both targeting the proteins to the membrane and their subsequent translocation across the membrane. A molecular machine called the translocase recognizes the secretory proteins and assists in their travel across the membrane (Fig.1).

Once translocated across the inner membrane, the secretory pre-protein is tethered to the membrane by the signal peptide. A membrane bound endo-peptidase, called signal or leader peptidase, functions to cleave off the signal peptide, thereby releasing the mature secretory protein from the membrane and allowing it to proceed to its final destination. Although the crystal structure of the catalytic domain of the signal peptidase has been solved in our laboratory (Paetzel et al., 1998) and numerous spectroscopic studies (NMR, EPMR, and CD) have been performed on synthetic signal peptides in model membrane systems, the actual environment in which signal peptide cleavage occurs (i.e. within the membrane or not) still remains unclear. We present here a brief analysis of known features of the signal peptide, signal peptidase and bacterial membrane structure and the resultant implications for the likely location of the scissile bond cleavage.

Signal peptide primary structure

In general, signal peptides share little sequence identity, but they do contain some common features in size and distribution of electrostatic and hydrophobic residues (Fig. 2). All have a short, positively charged amino-terminal region (n-region, 1-5 residues), a longer hydrophobic middle region (h-region, 7-15 residues), and a carboxy-terminal protease recognition region (c-region, 3-7 residues). The protease recognition region contains small aliphatic or polar residues at the -1 and -3 positions relative to the cleavage site (or P1 and P3 in the Schechter and Berger (1967) nomenclature). Alanine is most often observed at the -1 and -3 positions (von Heijne, 1983, 1985; Perlman & Halvorson, 1983, Fig. 2). The -6 position of the signal peptide is most often occupied by a Pro, Gly or Ser residue and it has been suggested that this position defines the transition from the hydrophobic h-region to the c-region (von Heijne, 1990). There are a number of computational methods for identifying signal peptides and their cleavage sites from the sequence of proteins. One of the most popular and accessible is the SignalP World Wide Web server (<http://www.cbs.dtu.dk/services/SignalP/>), which is based on a combination of sev-

eral artificial neural networks (Nielsen et al., 1997a,b). The signal peptides from gram-positive bacteria are significantly longer than those from other organisms, and they have a much longer h-region (von Heijne & Abrahmsen, 1989). The average eukaryotic signal peptide is 22.6 amino acids in length, the average gram-negative signal peptide is 25.1 amino acids in length, and the average gram-positive signal peptide contains 32.0 amino acids (Nielsen et al., 1997a,b). The gram-positive signal peptides in general have an amino-terminus containing more lysine and arginine residues (Edman et al., 1999). The positively charged n-region is thought to play a role in the proper orientation of the signal peptide in the lipid bilayer.

Signal peptide secondary structure

A number of studies have focused on the structure, orientation, and interactions of signal peptides in lipid bilayers or membrane mimetic environments (Cornell et al., 1989; Bruch et al., 1989; Jones et al., 1990; McKnight et al., 1991a,b; Wang et al., 1993; Rizo et al., 1993; Jones & Gierasch, 1994; Bechinger et al., 1996; Keller et al., 1996; Voglino et al., 1998; Voglino et al., 1999). Most studies to date are consistent with the central h-region adopting an α -helical conformation when in a lipid or hydrophobic environment (McKnight et al., 1989; Chupin et al., 1995; Voglino et al., 1998; Batenburg et al., 1988; Rizo et al., 1993; and Wang et al., 1993). The boundary between the h-region and the c-region (-6 to -4), which often contains proline or glycine residues, has been suggested to

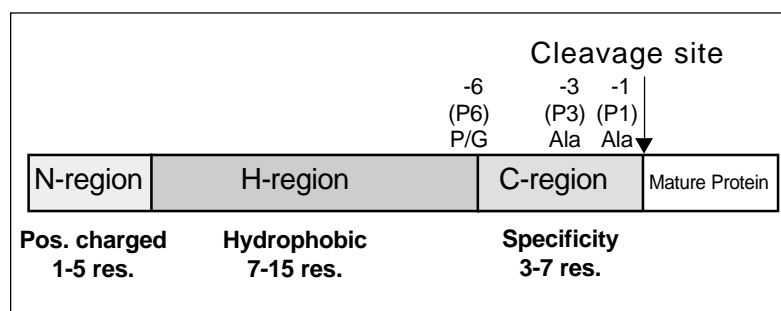


Figure 2. The features of a typical bacterial signal peptide. Signal peptides have a positively charged amino-terminus (n-region), a hydrophobic central region (h-region), and a neutral but polar carboxy-terminus (c-region). The boundary between the h-region and the c-region is usually marked by a helix-breaking residue (Pro or Gly) at the -6 (P6) position relative to the cleavage site. The cleavage recognition sequence consists of small residues at the -1 (P1) and -3 (P3) positions relative to the cleavage site. By far the most common residues at these positions is alanine.

have a β -turn structure (Rosenblatt et al., 1980; Perlman & Halvorson, 1983). The recent conformational, statistical and mutational analysis by Karamyshev and coworkers (1998) is consistent with the signal peptide having an extended β -conformation in the -5 to -1 region while bound to the signal peptidase binding pocket.

Signal peptidase structure

Escherichia coli type I signal peptidase is by far the most thoroughly studied signal peptidase. Typical of many gram-negative species, *E. coli* signal peptidase (323 amino acids, 35,988 Da) has two predicted amino-terminal transmembrane segments (residues 4-28 and 58-76), a small cytoplasmic domain (residues 29-57), and a large carboxy-terminal catalytic domain (residues 77-323) (Fig. 1, 3).

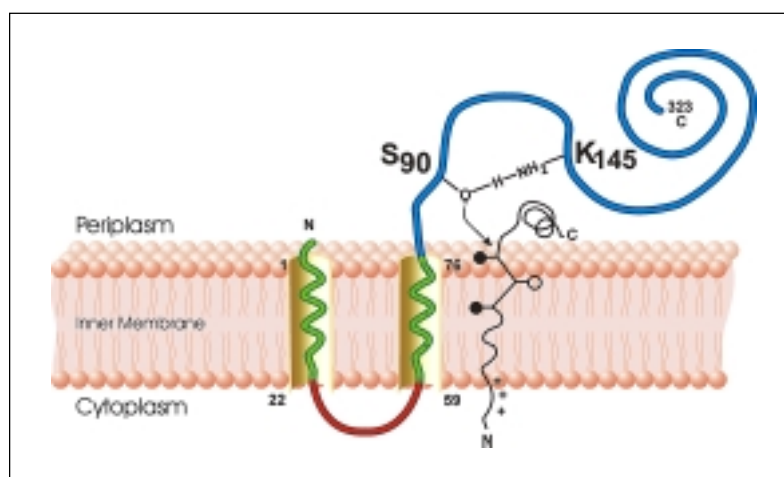


Figure 3. The membrane topology of *Escherichia coli* signal peptidase.

The crystal structure of the catalytic domain of *E. coli* signal peptidase has been solved in our laboratory (Paetzel et al., 1998). Although the N-terminal membrane anchor was deleted in the construct used for the structure determination, appropriate concentrations of detergent were still required for optimal activity (Tschantz et al., 1995) and for crystallization (Paetzel et al., 1995). The structure revealed for the first time the catalytic residues (a Ser/Lys dyad) and substrate binding site of a signal peptidase. The extended, shallow hydrophobic binding cleft is consistent with the observed substrate specificity and prediction of an extended beta-conformation for the c-region of signal peptides (Fig. 4). Also observed is an unusually extensive exposed hydrophobic surface that runs

along the full length of the central β -sheet and includes the hydrophobic substrate binding site. Exposure of significant areas of hydrophobic surface is rarely observed in soluble proteins and we proposed this unusual surface would be involved in membrane association (Paetzel et al., 1998). Located on this predicted membrane association surface are a number of aromatic residues, including Trp 300, which was shown to be essential for optimal activity in *E. coli* signal peptidase (Kim et al., 1995a,b), even though our structure maps it to a position more than 20 Å from the enzyme catalytic center (Paetzel et al., 1998, Fig. 4). Aromatic amino acids are thought to play an important role in protein/membrane interfaces (Landolt-Marticorena et al., 1993) and presumably Trp 300 facilitates the insertion or association of *E. coli* signal peptidase into the membrane. A tryptophan residue was also found to be essential for the interfacial catalysis of phospholipase A_2 at the membrane surface (Gelb et al., 1999). Sequence alignments indicate that several conserved aromatic or hydrophobic residues exist in the proposed membrane-association domain in both gram-positive and gram-negative bacterial type I signal peptidases.

Lipid membrane structure

X-ray and neutron diffraction data on fluid phospholipid bilayers (dioleoylphosphatidylcholine) show that the hydrocarbon core (the hydrophobic fatty acid chains) of the bilayer is approximately 30 Å thick and that the interfacial region (the phospholipid head groups and glycerol-fatty acid ester backbone) is approximately 15 Å on each side (Wiener & White, 1992, Fig. 5).

Even though the overall bacterial membrane is in a bilayer (lamellar) structure, it is essential for the membrane to be near to the bilayer-nonbilayer transition point. This is achieved by organisms maintaining a certain proportion of phospholipid in their membrane that have a strong preference for nonbilayer states. These phospholipids have a small head group compared to their acyl-chains that give them an overall conical shape. One of these nonbilayer phospholipids is phosphatidylethanolamine. The *Escherichia coli* inner membrane is made up of approximately 75 % phosphatidylethanolamine.

Evidence for the insertion of the catalytic domain of signal peptidase into the membrane

Using the catalytic domain of *E. coli* signal peptidase (as described in our crystallographic study) van Klompenburg and colleagues showed, using monolayer and vesicle binding experiments, that despite the absence of the transmembrane segments, the signal peptidase catalytic domain penetrated deeply into lipid monolayers with a marked preference for the nonbilayer lipid dioleoylphosphatidylethanolamine (van Klompenburg et al., 1998). Recently this preference for nonbilayer lipid has been shown to be independent of the exact chemical structure of the lipid head group (van den Brink-van der Laan et al., 2001). It is interesting that NMR (Killian et al., 1990) and ESR (Sankaram et al., 1994) experiments implicate the signal peptide in promoting non-bilayer lipid structure (deVrije et al., 1990) and non-bilayer lipid structure as important for proper function of the translocase (Rietveld et al., 1995; van der Does et al., 2000).

Based on the evidence that the catalytic domain of *E. coli* signal peptidase penetrates into lipid membranes as well the extensive hydrophobic surface on the signal peptidase catalytic domain we have modeled the *E. coli* signal peptidase bound to a signal peptide. We have modeled the c-region of the signal peptide substrate in an extended conformation that can form beta-sheet hydrogen bonds with the beta-strands that line the binding site (typical of a many known proteases). The peptide substrate then transitions into the alpha-helical h-region, and finally to the n-region. The model is presented along side a pair of phospholipid molecules taken from a molecular dynamics simulation of a bilayer system (Heller et al., 1993). The location of the signal peptidase hydrophobic binding-site, the extended hydrophobic surface, as well as the location of the amino-terminus, gives us an approximate orientation of signal peptidase relative to the plane of the membrane. Given that the average gram-negative signal peptide is 25.1 amino acids in length, the helical, hydrophobic segment (h-region) would be at most 15 residues in length. Calculating the length of this helix using the typical values of 3.6 residues/turn and 5.4 Å in pitch estimates an overall length of 22.5 Å for the h-region. Clearly, in this model, the h-region of the signal peptide would be too short to span the aliphatic portion of the fatty acid chains of a typical lipid bilayer. Pre-

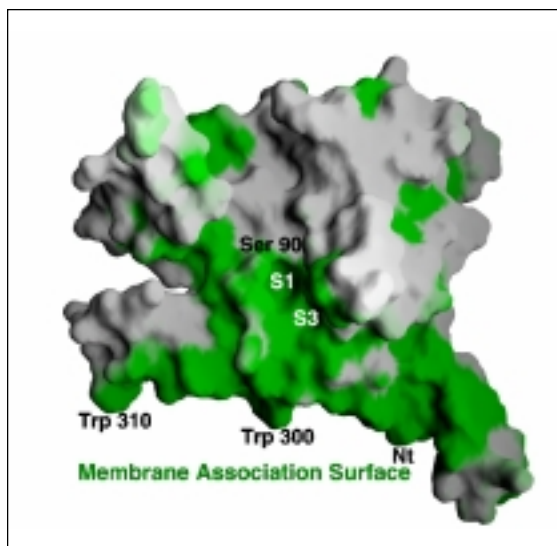


Figure 4. The extended hydrophobic surface on the catalytic domain of *Escherichia coli* signal peptidase.

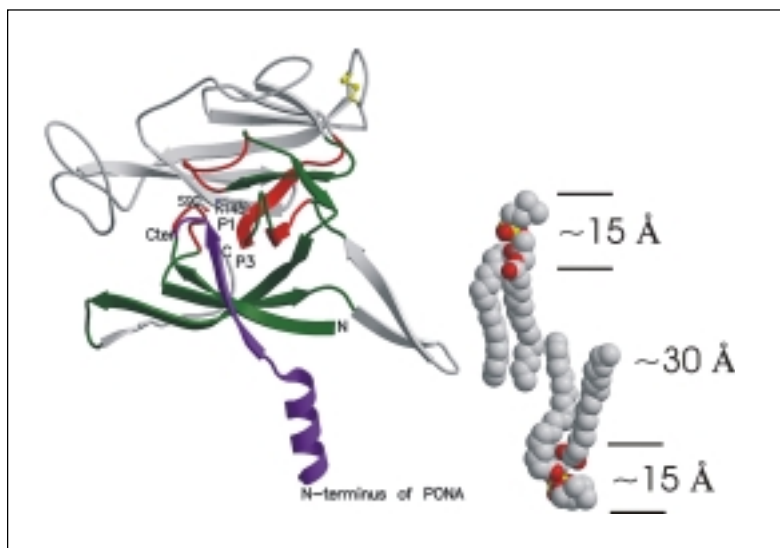


Figure 5. A model of a signal peptide bound to the catalytic domain of *Escherichia coli* signal peptidase. The dimensions of a typical lipid bilayer are shown adjacent to the model.

suming the positively charged n-region of the signal peptide keeps the amino-terminus on the cis-side (or cytoplasmic side) of the inner membrane, we predict the cleavage-site of the signal peptide would reside in the hydrophobic acyl-chains of the fatty acids or at the more polar region of the glycerol backbone of the phospholipid (Fig.5).

Future Experiments

To date there is no direct experimental evidence to confirm whether the bacterial signal peptide is associated with lipid and/or the proteins of the secretion machinery (translocase) during the cleavage event *in vivo*. It is also presently unclear whether bacterial signal peptidases cleave the signal peptide during or after translocation and in what phase the membrane lipids exist at the time and place of protein translocation. These are obviously questions that need to be addressed experimentally in order to further refine our view of the essential cleavage of signal peptides in bacteria and higher order species. Work is now underway to solve the crystal structure of the full-length *E. coli* signal peptidase in complex with a signal peptide. Solid state NMR experiments will be performed to investigate the structure of the signal peptidase transmembrane segments and the signal peptide in a lipid environment and EPR and fluorescence experiments will be utilized to measure the depth of the insertion by the catalytic domain of *E. coli* signal peptidase.

ACKNOWLEDGEMENTS:

This work was supported by the Canadian Institute of Health Research, the Canadian Bacterial Diseases Network of Excellence, the Burroughs Wellcome Foundation and the Howard Hughes Medical Institute grants to NCJS. M.P. is supported by a CIHR post-doctoral fellowship and NCJS by a CIHR Scholarship. We thank our collaborators and colleagues who have provided advice, mentoring and funding opportunities that have helped our new laboratory to flourish. George Mackie, Susan Jensen, Brett Finlay, Ross E. Dalbey, John Little, Martin Tanner, Stephen Withers, Joel Weiner, Robert Hancock, Warren Wakarchuk, Ross MacGillivray, Grant Mauk, Carolyn Astell, Roger Brownsey, Lawrence Mcintosh, Victor Ling, Julian Davies, Glen Armstrong – thank you!

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