FOR THE RECORD

Crystallization of the chaperone protein SecB

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Abstract: The secretory protein SecB found in *Escherichia coli* is a molecular chaperone that binds to precursor forms of a number of proteins targeted for export to the periplasmic space. SecB maintains these proteins in a translocation-competent conformation facilitating the translocation process. The material has been cloned and expressed in *E. coli*. Crystals have been grown from polyethylene glycol 8000 by vapor diffusion using the hanging drop technique. These crystals are monoclinic, belonging to space group C2 with unit cell dimensions $a = 56.0$ Å, $b = 111.1$ Å, $c = 134.7$ Å, and $\beta = 104^\circ$. The crystals diffract to 8 Å resolution on a Rigaku imaging plate detector. Dynamic light scattering experiments suggest that SecB exhibits aggregation behavior with a number of different precipitating agents. These results may explain resistance of SecB to forming ordered crystals.

Keywords: crystallization; molecular chaperone; protein translocation; SecB

Proteins that are translocated across biological membranes to become localized in extracytoplasmic locations are found in precursor forms containing an N-terminal signal peptide that is cleaved after translocation. These signal peptides are believed to be responsible for stabilizing export-competent conformers of the precursor proteins (Collier et al., 1988; Crooke et al., 1988; Kumamoto & Gannon, 1988) and assist in targeting them to further protein components along the translocation pathway. The cell machinery responsible for protein translocation to the periplasmic space in *Escherichia coli* recognizes the precursor form of proteins targeted for translocation and carries them to the membrane where they are inserted and translocated in an ATP-dependent fashion.

The secretory "chaperone" protein, SecB, found in *E. coli*, participates in the early steps of the translocation pathway by binding to the precursor forms of a number of exported proteins such as maltose binding protein (MBP) (Weiss et al., 1988). SecB carries the precursor proteins to SecA, an ATP-dependent component of the translocation pathway (Hartl et al., 1990). SecA interacts with the integral membrane proteins SecE and SecY

and, upon ATP hydrolysis, facilitates insertion of the precursor protein in the membrane (Brundage et al., 1990). SecB is found as an acidic, soluble, oligomeric protein of 16-kDa subunits (Weiss et al., 1988; Kumamoto et al., 1989; Watanabe & Blobel, 1989). It binds to segments both within the signal peptide sequence and within the mature moiety of precursor proteins (Watanabe & Blobel, 1989). However, results show that SecB specifically recognizes non-native or unfolded structures (Randall et al., 1990). This suggests that the role of the signal peptide may be to slow down the folding of the precursor relative to the mature protein thus prolonging the lifetime of the non-native states and facilitating the interaction with SecB (Park et al., 1988; Liu et al., 1989; Teschke et al., 1991).

A crystallographic determination of the structure of SecB will enable a detailed understanding of the mechanism by which this molecular chaperone recognizes precursor proteins with such a broad specificity. In addition, the structure will direct further studies aimed at understanding the mechanism by which SecB modulates the folding pathway of precursor proteins.

Results: SecB was cloned into the plasmid PJW25 and over-expressed in *E. coli* strain BL21 (Kumamoto et al., 1989). The purification protocol was slightly modified from the procedure of Weiss et al. (1988) in order to obtain material of suitable quality for crystallization experiments. The cell pellet was lysed as described by Weiss et al. (1988) except that EDTA was omitted from the protocol. The lysate was dialyzed against 20 mM Tris, pH 7.4, in 0.1 mM PMSF. The dialyzed lysate was applied to a Q-Sepharose ion-exchange column and washed with 500 mM NaCl in 20 mM Tris, pH 7.4. The flow through was collected, dialyzed again 20 mM Tris, pH 7.4, and 0.1 mM PMSF, and concentrated by Amicon (PM10 membrane) to 12–15 mg/mL total protein. The material was then rechromatographed on an ion-exchange HR10/10 MonoQ FPLC column eluting with a gradient of 0–500 mM NaCl in 20 mM Tris, pH 7.4. SecB was eluted at 250 mM NaCl. The fractions containing the protein were initially concentrated by Amicon and further concentrated and dialyzed by ProDiMemCon (Biomolecular Dynamics) against 25 mM Na-phosphate and 100 mM Na₂SO₄, pH 7.0, to 16 mg/mL. The concentrated protein was applied to a TSK-3000SW gel-filtration HPLC column and eluted at a flow rate of 3 mL/min with 25 mM Na-phosphate and 100 mM Na₂SO₄, pH 7.0. The fractions containing SecB were initially concentrated by Amicon and further concentrated and dialyzed by ProDiMemCon into 20 mM Tris, pH 7.4.

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Crystallization trials were carried out by vapor diffusion using the hanging drop method. Initial trials used a sparse matrix biased toward known crystallization conditions (Jancarik & Kim, 1991) and resulted in small crystals from polyethylene glycol (PEG) 8000. Refinement of these conditions gave crystals that appear after 7 days using a well solution of 10–12% PEG 8000, 100 mM CaCl_2 , 10 mM β -mercaptoethanol, 100 mM NaCl, and 100 mM Hepes, pH 6.5–7.7. Crystals also appear in the absence of NaCl and β -mercaptoethanol; however, the absence of CaCl_2 from the crystallization conditions resulted in highly disordered crystals. The drop contains equal volumes of the well and 10 mg/mL SecB in 20 mM Tris, pH 7.4, and 10 mM β -mercaptoethanol (see Fig. 1).

The space group was determined by precession photography to be P2 with cell dimensions $a = 56.0 \text{ \AA}$, $b = 111.1 \text{ \AA}$, $c = 134.7 \text{ \AA}$, and $\beta = 104^\circ$. The crystals diffract to 8 $\text{\AA}$ resolution on a Rigaku imaging plate detector and attempts at the synchrotron radiation facility (Stanford) gave diffraction to approximately 4.5 $\text{\AA}$. However, the crystals die after only a single exposure.

In an attempt to improve the quality of the SecB crystals or grow a new form that diffracted better, we used dynamic light scattering (DLS) to monitor the behavior of the protein in the presence of a variety of common crystallization precipitants. We performed a series of simple experiments based on the approach suggested by Ducruix and Giege (1992), where the protein concentration was held constant and the precipitant concentration varied. This allows the diffusion coefficient of the protein to be determined as a function of the precipitant concentration. The idea behind this approach is to rule out the potential crystallization conditions where aggregation occurs prior to reaching supersaturation. This type of behavior has been interpreted as nonspecific aggregation that will prevent molecules from forming a crystalline lattice under supersaturating conditions where crystallization would normally begin.

Our results are summarized in Figure 2. As a control, we monitored the behavior of lysozyme at 2 mg/mL in the presence of two precipitants, NaCl and MgSO_4 . Lysozyme has been successfully crystallized from NaCl (Mikol et al., 1990); however,

crystals of lysozyme have never been obtained from sulfate salts (Mikol et al., 1990). Our results are consistent with this behavior. Figure 2A shows that, in the presence of NaCl, the apparent molecular weight (MW) of lysozyme remains constant until concentrations approaching those where crystallization occurs are reached. In contrast, with MgSO_4 , the apparent MW of lysozyme begins to increase almost immediately. SecB was tested at 2 mg/mL in the presence of 14 common precipitants, and our results fall into three general categories. First, reliable DLS measurements could not be obtained from a number of precipitants including all of the PEGs and NH_4 sulfate. This may be due to rapid aggregation of the protein.² Second, with several precipitants, the aggregation behavior of SecB resembled that of lysozyme in the presence of Mg sulfate (Fig. 2B). Finally, in the case of NH_4 nitrate and methane pentanediol (MPD), the apparent MW of SecB decreased with increasing precipitant (Fig. 2C). We are uncertain how to interpret this category of results. In the absence of other data, it would suggest that the SecB tetramer is not stable under these conditions. The DLS results are consistent with the apparent resistance of SecB to forming ordered crystals.

Another factor influencing the nature of the SecB crystals may be related to an understanding of the biological function of chaperonins. It is known that SecB exhibits a broad range of substrate specificity, interacting with precursor forms of a number of different and supposedly unrelated proteins. Although the features defining a SecB binding site are not yet known, it is suggested that the specificity for binding is low (Randall & Hardy, 1995). It is thought that SecB binds to precursor proteins and stabilizes them in a prefolded conformation through several low-affinity sites (Kumamoto, 1989; Lecker et al., 1989; Watanabe & Blobel, 1989; Randall et al., 1990). These low-affinity sites may consist of a number of flexible portions or hydrophobic areas. The leader peptide of the precursor proteins acts to retard the folding and thus expose these low-affinity

² Because of their relatively high MW and effect on sample viscosity, the polymeric precipitants such as PEG may influence the diffusion coefficient (and apparent MW) calculated for SecB as determined by DLS.

Fig. 1. Crystals of SecB grown by vapor diffusion using the hanging drop method. Crystal size is approximately $0.4 \times 0.3 \times 0.15 \text{ mm}$.

Fig. 2. A: Plot of the apparent MW of lysozyme (estimated from the diffusion coefficient determined by DLS) versus the salt concentration of either NaCl or MgSO₄. Note the lack of aggregation below 1 M NaCl. B, C: Plot of the apparent MW of SecB versus the concentrations of two representative precipitants.

binding sites for recognition and binding by SecB (Liu et al., 1989; Randall et al., 1990). The nonspecific nature of each of these recognition sites suggests that SecB may exhibit a wide range of mobile conformations, enabling it to recognize different precursor proteins. It is expected that this suggested mobile nature for SecB would cause problems in the formation of a stable crystal lattice and may explain the poor diffraction quality of the protein. It may be suggested that, in order to minimize this mobility and improve the crystal quality, a complex be formed between SecB and a precursor protein. This interaction may hold SecB in a conformation that will decrease the potential mobile nature of regions of the chaperone and may facilitate the growth of crystals more suitable for X-ray diffraction experiments. Attempts to improve the quality of these crystals have not yet been successful.

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