Analysis of Complex Bacterial Cell Division Proteins by Size Exclusion Chromatography (SEC)

Application Note

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Introduction

Globular proteins are so named because they have roughly spherical shapes in their native forms. However, many proteins operate not as isolated entities but as part of larger structures, for example many proteins are components of cell walls. Identifying and isolating the native proteins from these complex systems can be challenging. Analysis of proteins by SEC is a powerful tool for investigating the structure and aggregation of proteins. By separating proteins on the basis of size in solution, SEC can be used to identify and isolate proteins from such complex systems.

This application note demonstrates the analysis of FtsZ and ZipA, two essential cell division proteins involved in septum formation in Escherichia coli (E. coli). Crystal structures of the two protein monomer units are shown in Figures 1 and 2. FtsZ is a major component of the bacterial septal ring and the first protein to move to the division site during cell fission. FtsZ is responsible for binding other proteins that go on to produce a new cell wall between the dividing cells, an example of which is the protein known as ZipA.

Reconstituting the membrane-anchoring protein ZipA on E. coli lipid membranes now provides information on how membrane attachment regulates FtsZ polymer dynamics. The interaction of ZipA with the membrane greatly increases the size of the protein conjugate allowing easy separation from the native unattached protein, so SEC allows the confirmation of encapsulation of the protein within the lipid bilayer.

In this study FtsZ and ZipA were analyzed by SEC.

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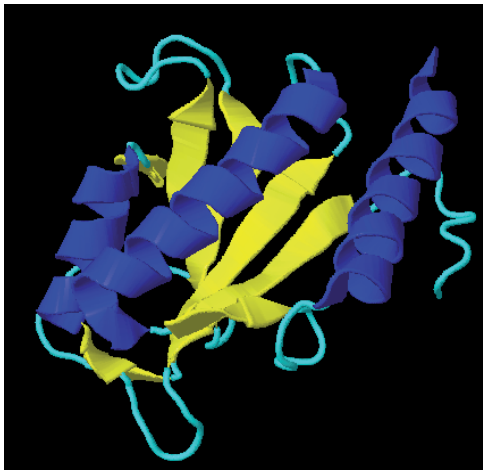

Figure 1. Crystal structure of ZipA composing α-helices and a β-sheet. The N-terminus is a predominantly hydrophobic transmembrane domain that acts as an anchor for the docking of the protein to the cytoplasmic domain. The C-terminal domain is responsible for the binding of ZipA to FtsZ. (http://bionmr-c1.unl.edu)

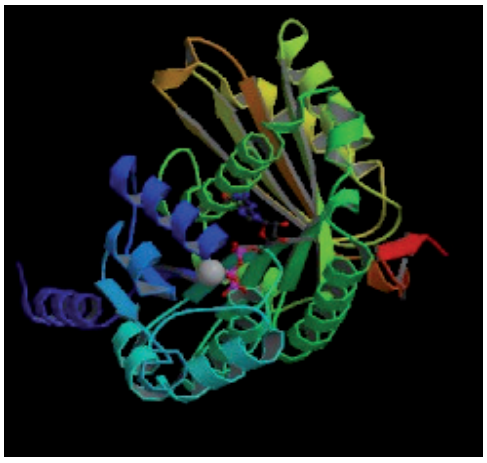

Figure 2. Crystal structure of FtsZ comprising a mixture of α-helices and β-sheets. The N-terminus is the GTPase domain whilst the C-terminus is required for binding to other proteins such as ZipA. (http://www.ionchannels.org/)

Methods and Materials

Conditions

Column:	Agilent ProSEC 300S, 300 x 7.5 mm (p/n PL1147-6501)
Flow rate:	1 mL/min
Temperature:	5 °C
Detection:	UV at 280 nm
Injections:	ZipA-100 mL @ 0.075 mg/mL conc. FtsZ-100 mL @ 1.2 mg/mL conc.
Sample:	ZipA and FtsZ
Eluent:	0.1 M KH_2PO_4 containing 0.3 M NaCl, pH 8.0

Results and Discussion

Figure 3 shows a UV chromatogram for a sample of ZipA and FtsZ obtained on the ProSEC 300S column.

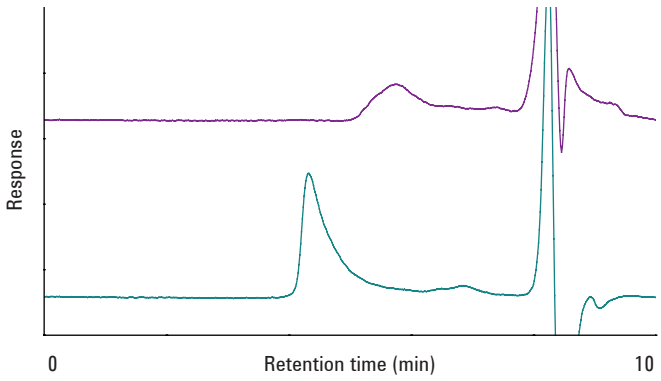

Figure 3. UV detector chromatogram for an example of ZipA and FtsZ

The ZipA sample eluted predominantly as a single excluded peak at 5.3 minutes and a very small subsidiary peak at 8 minutes. The peak at 5.3 minutes represents lipid bilayer embedded protein that has been excluded from the column due to its very large size, while the peak at 8 minutes illustrates the presence of a protein of approximately 50 kDa, which corresponds to ZipA embedded in the lipid bilayer. The ProSEC 300S column is therefore suitable for isolation of unencapsulated protein from the lipid-protein conjugate, allowing the complex to be purified for further studies.

The FtsZ sample eluted as a broad peak centred at 7 minutes, indicating an impure protein of approximately 50 kDa.

Conclusion

The results show that SEC can be used for the identification of proteins that have been encapsulated in a lipid bilayer from those that are not. Assessing the protein molecular weight using SEC allows the identification of successful lipid encapsulation of proteins and polymeric species. Confirmation of polymer formation will allow the progression of studies into the effect of these polymers on bacterial cell division.

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