

Production of FtsZ protein as a precursor to investigating the molecular mechanism of FtsZ fibre formation. Nigel Dyer. Supervisors Dr. David Roper and Prof. Alison Rodger.

Abstract

The prokaryotic protein FtsZ was expressed and purified and some initial experiments performed to verify its activity. This protein will be used to investigate its role in prokaryotic cell division.

Introduction

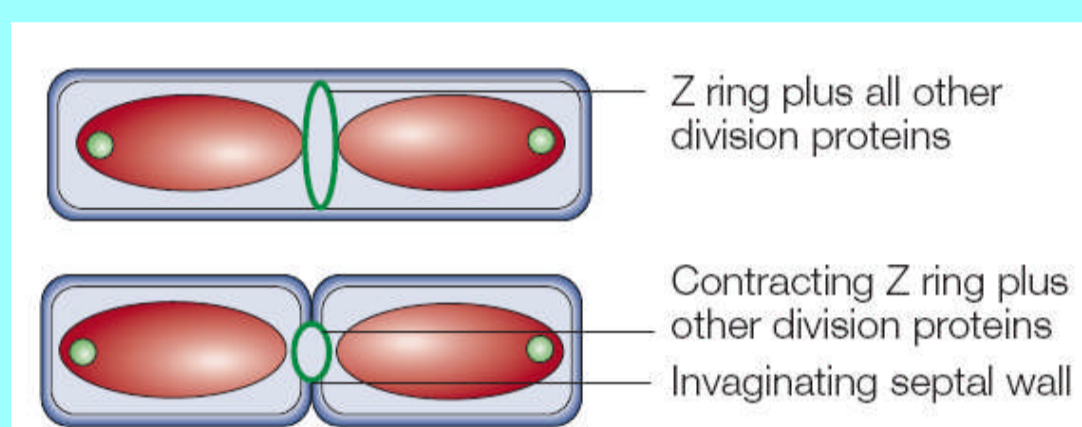
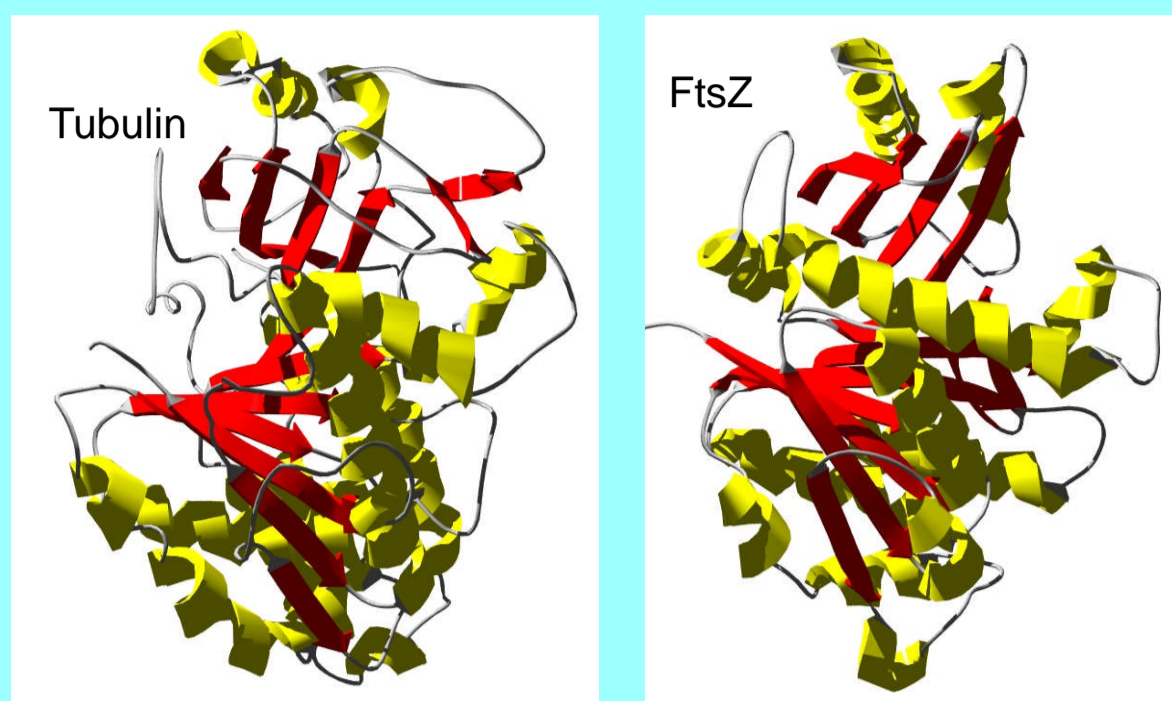
Prokaryotes contain FtsZ, a homologue of eukaryotic tubulin.

Both form filaments, are critical to cell division, form dynamic, spatially well defined structures and bind and hydrolyse GTP to stabilise their filament forms.

FtsZ forms a z-ring around the middle of prokaryotic cells (the divisome) to split the cell during cell division.

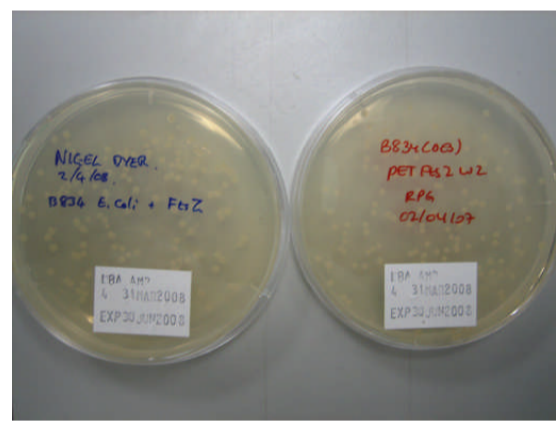
FtsZ works with a dozen or so other proteins which stabilise the Z-band and link the band to the bacterial cell wall, including ZipA, FtsA and especially YgfE (a homologue of ZapA) subunits which are all associated with early ring formation and bundling.

The interaction between FtsZ and its partners is still poorly understood. This objective of the mini-project was to produce some FtsZ protein so that Linear Dichroism and other biophysical techniques could be used to explore the dynamic interaction and relative orientation of these proteins during the process of cell division.



Method and Results: FtsZ production.

1. FtsZ expressed in B834 (DE3) *E. coli*.



2. Two colonies transferred to two 15 mL LB media and, after incubation, split between 12 litres of media. *E. coli* expression was induced by adding IPTG, when an optical density of 0.4 was reached.



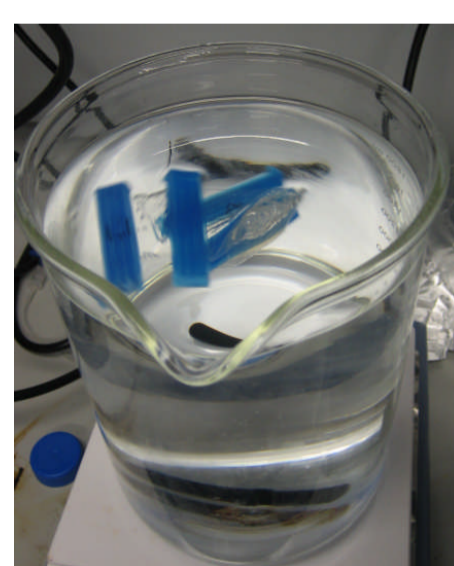
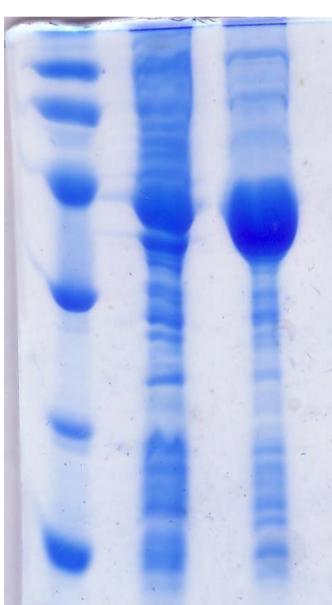
3. After 3 hours, the cells were harvested by centrifuging, and re-suspended in Buffer A

The cells were lysed by sonication, and centrifuged to remove all but the soluble proteins.



4. The FtsZ was precipitated out using 30% ammonium sulphate, centrifuged and re-suspended in Buffer A and dialysed to remove excess salts

SDS page column 2 shows proteins present before precipitation 3 shows proteins present afterwards, indicating the extent of protein purification achieved at this stage.



5. Ion exchange chromatography using an DEAE-separate column was used to separate the FtsZ. Two batches were processed; the first from one litre of cell culture, the second from four litres. Absorbance at 280 nm was used to monitor for protein in the elute.

Results showed a peak at 0.3 M KCl running into a second at 0.35 M KCl.



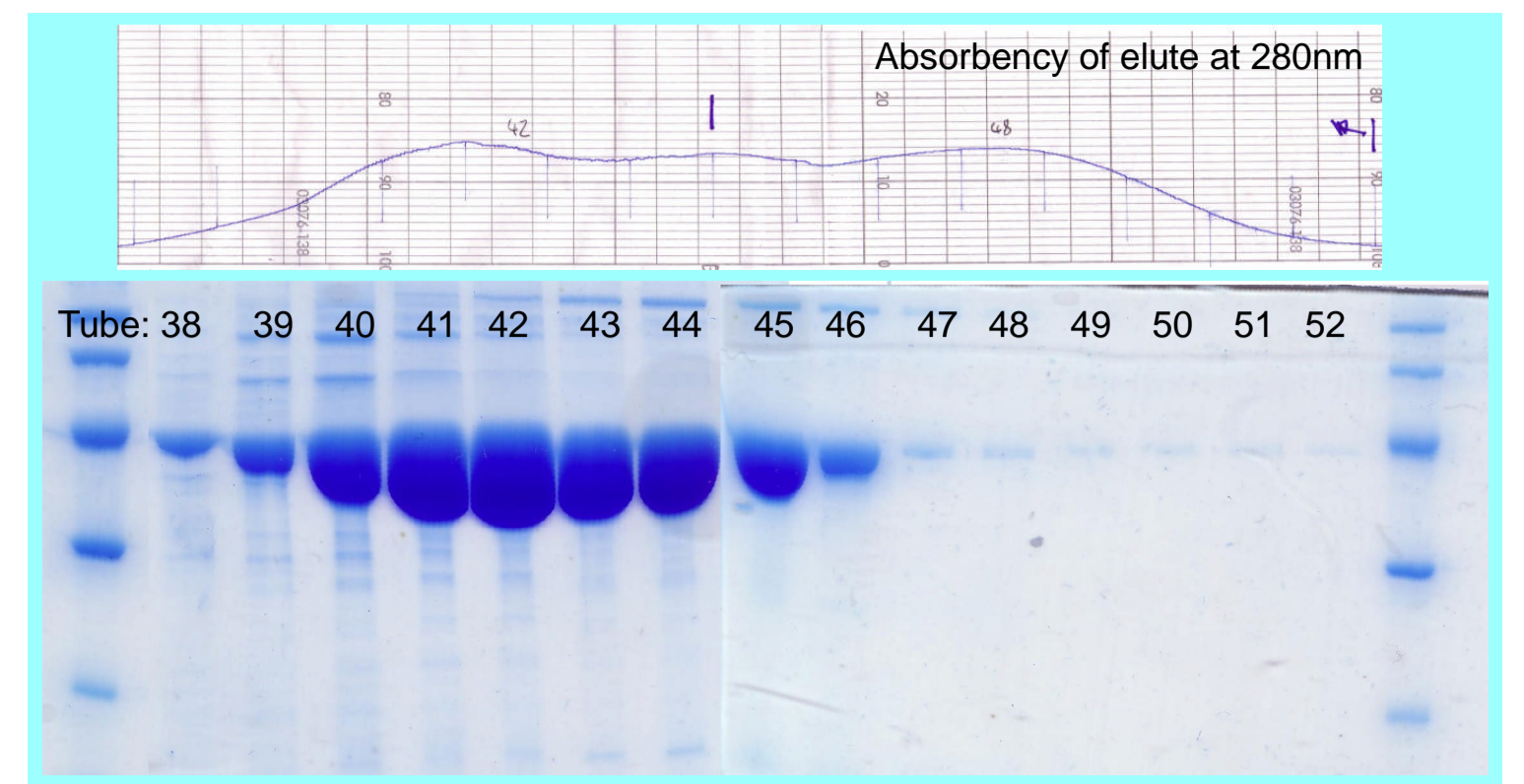
Buffer A: 50 mM KCl, 50mM Tris, 1 mM EDTA and 10% glycerol, the latter because it has been found to keep FtsZ in a monomeric form.

Acknowledgements

I would like to thank my supervisors Dr. David Roper and Prof. Alison Rodger. Also Andrew Quigley, Jenni Shepherd, Kat Abrahams, Ian Portman, Martyn Rittman and others who provided additional support in the lab, and above all Raul Pacheco-Gomez who taught me so much in a very short time.

6. SDS-page gels shows significant protein associated with the first peak, and none with the second.

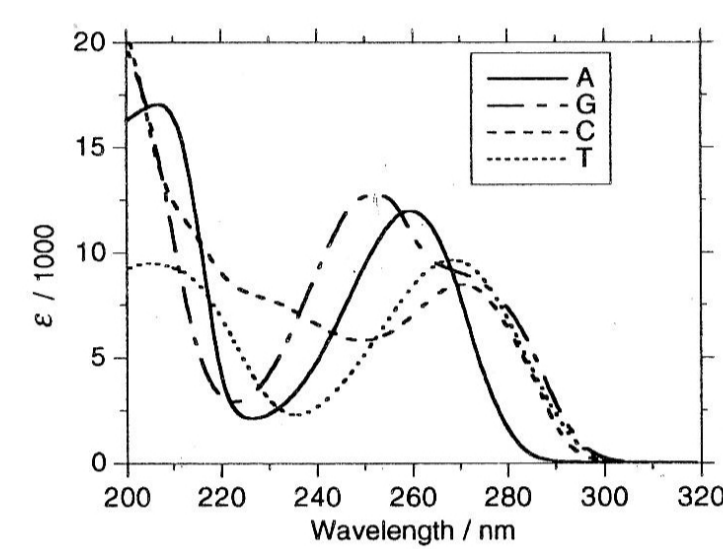
Similar results were obtained for both batches of FtsZ, prompting an investigation into the cause of the second UV absorbance peak centred around tube 48.



7. UV absorbance spectra of elute from tubes 40 and 48 were measured. These confirmed the original similar absorbencies at 280nm.

Results from tube 48 are characteristic of nucleotide absorbance.

The λ_{max} of 256 nm is midway between ATP (260) and GTP (253).



From: "Circular Dichroism and Linear Dichroism" 1997 Alison Rodger and Bengt Norden, Oxford Chemistry Masters.

However, the spectral shape suggests ATP.

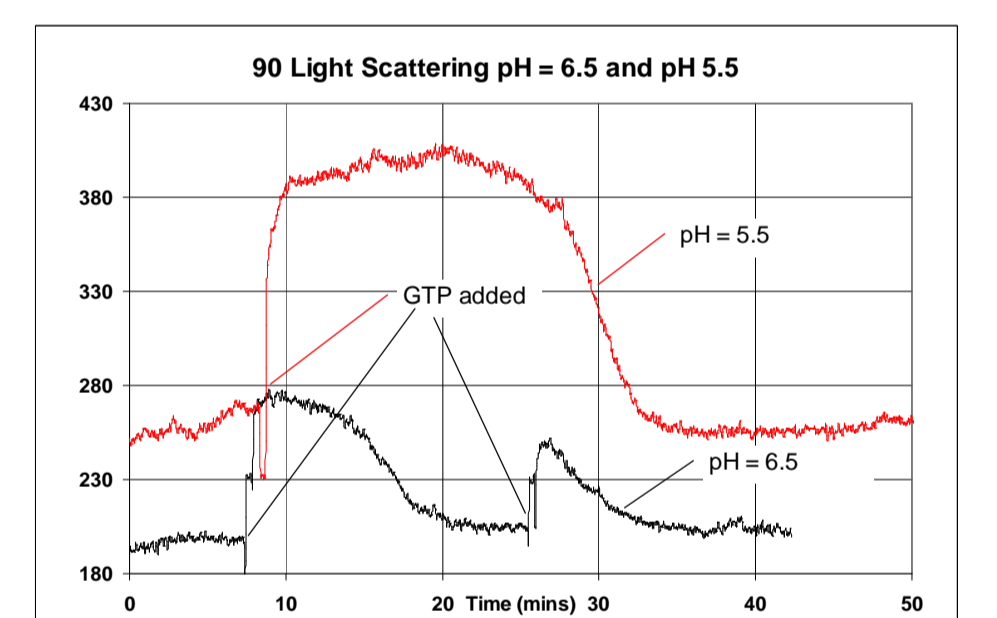
It might have been expected that some purified FtsZ might include bound GDP as this, and this would bind to the column and come off as a separate fraction. It is less clear why ATP should be present. This may be investigated further in the second mini project if there are suggestions that this may be important.

8. Four tubes of elute with maximum FtsZ concentration were selected from each batch, dialysed to remove excess KCl and stored at -80°C . Bio-Rad tests indicate that batch A produced 32 mg FtsZ from 1 litre of cells and batch B produced 77 mg of FtsZ from 4 litres of culture.

Verification of FtsZ activity.

9. Initial tests using 90° light scattering showed that GTP induced filament formation at pH 6.5 which lasts about 5 minutes until all the GTP is hydrolysed by the FtsZ.

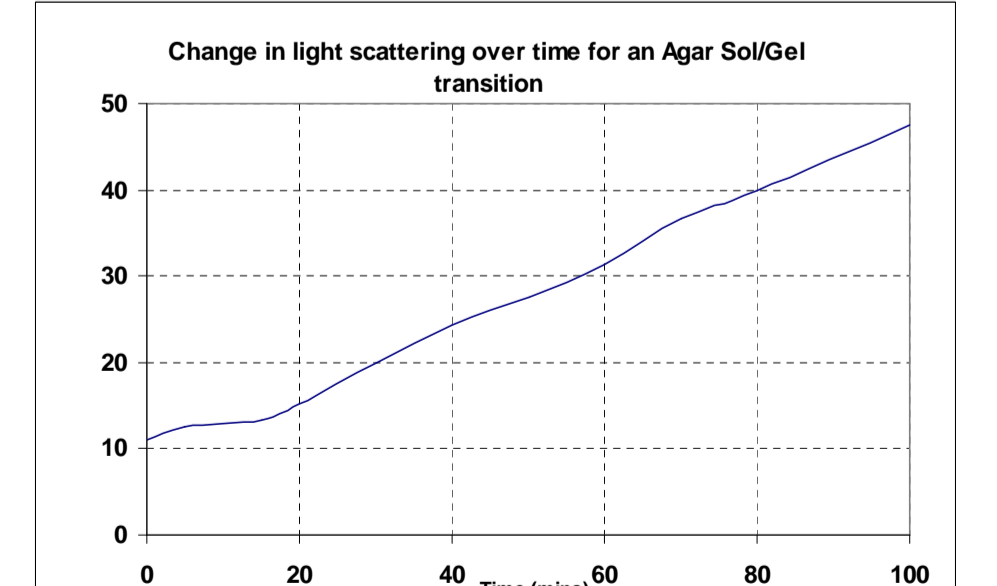
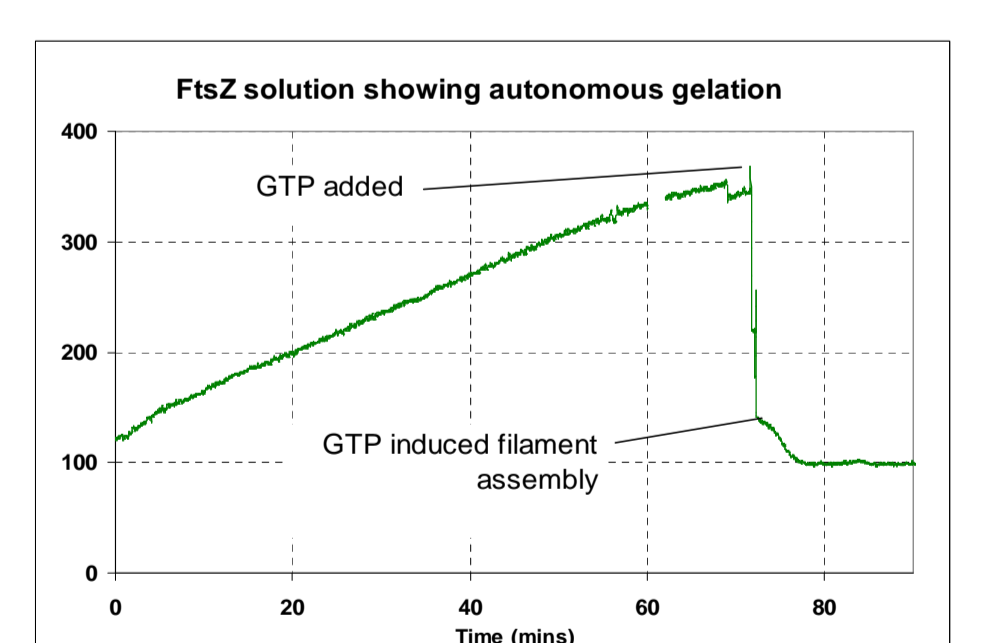
Similar tests at pH 5.5 indicate increased filament stability. This is consistent with previous results and confirms there is active protein.



10. A small change in the protocol resulted in a linear increase in light scattering for over an hour, which is characteristic of gel formation. Light scattering dropped on addition of GTP after which there was about 5 minutes of filament formation and then the light scattering returned to baseline. This is similar to previous tests and indicates that active FtsZ is still present.

This suggests that FtsZ may form meta-stable gels which might be an aspect of FtsZ filament stabilisation. The existence of meta stable gels may also explain the difficulties in obtaining repeatable results with FtsZ polymerisation studies.

This will be considered further in the second mini-project.



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